YEAST ASSIMILABLE NITROGEN SOURCE AND FERMENTATION TEMPERATURE AFFECTS THE CHEMISTRY AND SENSORY PROPERTIES OF COOL CLIMATE RIESLING

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ABSTRACT

Yeast-Assimilable Nitrogen (YAN) supplementation is common during commercial winemaking as it helps avoid stuck or sluggish fermentations and can improve wine quality. The majority of commercially available YAN supplementation products consist of inorganic nitrogen in the form of Diammonium Phosphate (DAP). Recent work has shown that the addition of organic nitrogen sources, such as autolyzed yeast nutrient or mixed amino acids, can confer additional benefits in terms of fermentation kinetics and volatile profiles. Further, fermentation temperature and nitrogen metabolism appear to have an interactive effect, meaning that nutrient packages may have varying outcomes at different fermentation temperatures. This work sought to add to the understanding of the impacts of nitrogen source and supplementation level by comparing the chemistry and sensory properties of wines supplemented with a range of inorganic versus organic nitrogen sources and at two different supplementation rates. Wine sensory attributes were assessed by a panel using a projective mapping technique known as Napping®. Wines that received the lower supplementation rate across several treatments were associated with more desirable sensory attributes.

A subsequent experiment examined the impact of fermentation temperature on the chemical and sensory outcomes of Riesling using inorganic or organic nitrogen

sources at three different fermentation temperatures. Fermentation temperature had a strong effect on fermentation kinetics, with warmer fermentation temperatures yielding faster fermentation. These wines were also evaluated by Napping®, and wines fermented at the lowest temperature were associated with more desirable sensory attributes.

BIOGRAPHICAL SKETCH

Seth Urbanek was born and raised in Houston, TX, and graduated in 2007 with a B.A. in International Relations and French from Texas A&M University. In 2008, Seth commissioned as an officer in the U.S. Army. Stationed out of Fort Drum, NY, Seth served for 26 months in Afghanistan in support of Operation Enduring Freedom, obtaining the rank of Captain. Seth was always planning to join the wine industry after his tenure in the Army, and in 2012, he began working for Sheldrake Point Winery in Ovid, NY. In 2015, Seth began his Master's in Food Science and Technology at Cornell University, concentrating on Enology. Under the supervision of Dr. Anna Katharine Mansfield, Seth researched the impacts of nitrogen supplementation source and fermentation temperature on wine quality of cool-climate Riesling.

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CHAPTER 1: LITERATURE REVIEW

During the course of fermentation of grape juice into wine, yeast nutrition is key to maintaining optimal yeast biomass and vitality. The nutrient that acts as a limiting factor during fermentation is nitrogen, specifically the forms that are available to yeast. Yeast Assimilable Nitrogen (YAN) comprises those nitrogenous compounds that can be metabolized by *S. cerevisiae*, and is defined as the sum of ammonia (AMM) and primary amino nitrogen (PAN). YAN does not include secondary amino acids, such as proline, or other polypeptides and proteins, as *S. cerevisiae* lacks the extracellular enzymes to degrade these compounds under anaerobic conditions (Querol and Fleet 2006). The composition of nitrogenous compounds found in grape must varies widely, containing a mixture of ammonium, protein, peptides, and amino acids. The resultant quantitative and qualitative variation of the grape must nitrogen content can affect both the kinetics of fermentation as well as the aromatic composition of the final wine (Querol and Fleet 2006).

Quantitative aspects of YAN are closely monitored in order to ensure healthy fermentation kinetics that minimize the risk of stuck or sluggish fermentations. A fermentation that starts slowly and remains sluggish throughout can often indicate a nitrogen deficiency. Additionally, fermentations that initiate normally then become sluggish can also indicate a nitrogen deficiency; these achieve maximal biomass, but have insufficient nutrient supply to maintain hexose consumption throughout the fermentation (Bisson 2000). If there is an intracellular nitrogen deficiency and a continual demand for amino acid/ protein synthesis, the yeast cell may begin to grow on sulfate in order to synthesize sulfur-containing amino acids, such as methionine and cysteine. This alteration of metabolic pathways can lead to production of hydrogen sulfide (H_2S), which is known to cause an unpleasant and foul aroma with very low sensory thresholds (Henschke & Jiranek 1995). Thus, in order to avoid poor fermentation kinetics and H_2S formation, sufficient YAN is required. Initial research indicated that 140 mg N/L was the minimum YAN level required to complete fermentation (Butzke 1998), yet the requirement may increase upwards of 250 mg N/L based on enological parameters such as yeast strain, sugar content, and

targeted fermentation time or aromatic composition. (Bisson & Butzke 2000). Excessive YAN supplementation during winemaking can also have negative impacts on ester production and sensory qualities of the final wine (Beltran et al. 2005, Torrea et al. 2011). In order to optimize fermentation kinetics and aromatic profile, the quantitative management of nitrogen is an important aspect of winemaking, as it allows the winemaker to ensure both the appropriate biomass and vitality of the yeast cells throughout fermentation.

Qualitative aspects of YAN, specifically the type of YAN supplied, can impact both the kinetics and aromatic composition of the wine as well. YAN enters the yeast cell via active transport, utilizing membrane transport proteins, known as permeases. Though all YAN can be transported by these permeases and subsequently metabolized, some sources are favored. Asparagine, glutamine, ammonium, and glutamate, due their central role in amino acid metabolism, are the most preferred nitrogen sources, in that order. Their utilization decreases the expression of genes for continued nitrogen uptake and catabolic enzyme levels, a change known as nitrogen catabolite repression (NCR) (Waterhouse et al. 2016, Jiranek et al. 1995). Large ammonium additions can induce NCR and inhibit the uptake of amino acids. This may have an impact on overall wine aromatics and quality, as amino acids serve as direct precursors for esters (Nykanen 1986).

YAN supplementation as part of commercial winemaking is a common practice, particularly in regions with consistently low must YAN. Riesling, in particular, has a higher proportion of amino nitrogen in the berry skin, which is often discarded during processing. Consequently, up to 30% of the berry YAN will be lost prior to fermentation, increasing the need to rely on nitrogen supplementation to the ferment (Stines et al. 2000). YAN supplementation, as part of fermentation, has shown to impact the production of volatile compounds, including fatty acids, fusel alcohols, and esters (Henschke and Bell 2005). Inorganic nitrogen is the form usually added to grape musts, typically in the form of Diammonium Phosphate (DAP). While inorganic ammonium is the preferred source for biomass formation, amino acids are preferentially used during the stationary phase for cell maintenance (Beltran et al. 2005). As

stated previously, DAP can induce NCR and decrease the uptake of amino acids, influencing volatile composition. Wines supplemented with organic nitrogen, in the form of amino acids, or with a combination of organic nitrogen and ammonium, have shown increases in acetate and ethyl esters, compared to wines supplemented solely with DAP (Torrea et al. 2011, Barbosa et al. 2012). Acetate esters are formed by the condensation of higher alcohols with acyl-CoA, and the concentration of higher alcohols is often inversely correlated with nitrogen additions, especially in moderate to high YAN concentrations (Vilanova et al. 2007, Carrau et al. 2008, Vilanova et al. 2012). Even when the concentration of higher alcohols is diminished due to nitrogen supplementation, there is a corresponding upregulation of the alcohol acyl transferases, ATF1p and ATF2p, which facilitate the formation of acetate esters. The concentration of higher alcohols is higher, however, in wines supplemented with a mixture containing amino acids, providing for increased substrate for this acetate ester formation (Garde-Cerdán et al. 2008, Ugliano et al. 2008, Torrea et al. 2011, Mouret et al. 2014). Lastly, nitrogen supplementation with amino acids combined with DAP has also been shown to increase medium-chain fatty acid (MCFA) and their corresponding MCFA-ethyl ester concentrations as well, the activity of which is modulated by which EEB1 and EHT1 enzymes (Torrea et al. 2011). As a result, nitrogen supplementation with amino acids can maximize the production of both acetate and ethyl esters.

Fermentation temperature can have a large impact on both nitrogen uptake and volatile aroma formation in the subsequent wine. The membrane permeases responsible for YAN transport can undergo structural changes based on temperature (Barnett and Barnett 1992). Both yeast biomass and nitrogen consumption increase at higher temperatures, and the changes in nitrogen uptake due to temperature are more pronounced when ammonium is the sole nitrogen source. NCR is less effective at permease repression at lower temperatures due to the decreased fluidity of the plasma membrane and permease functionality, meaning that low temperature fermentations are metabolically similar to low YAN fermentations (Beltran et al. 2007). While temperature can alter yeast nitrogen metabolism and volatile ester synthesis, temperature and nitrogen uptake can have an interactive effect. The length of the growth

phase of yeast does impact the total amount of volatile compounds produced, and the length of that growth phase is impacted by both initial nitrogen content and temperature. Higher temperatures result in faster kinetics, but higher initial nitrogen content can extend the length of the growth phase (Mouret et al. 2014). During the stationary phase, the activity of the enzymes responsible for acetate and ethyl ester formation are also affected by temperature. ATF1 and ATF2, responsible for acetate ester formation, are more active at lower temperatures, whereas those responsible for ethyl ester formation, EEB1 and EHT1, are more active at higher temperatures, particularly those above 18° C (Mouret et al. 2014). Due to the differences produced by varying quantitative and qualitative YAN supplementation as well as the interactive effect of temperature, this study focused on optimizing the fermentation parameters for Riesling in the Finger Lakes.

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CHAPTER 2:

INORGANIC AND ORGANIC YEAST ASSIMILABLE NITROGEN SOURCES IMPACT THE CHEMISTRY AND SENSORY PROPERTIES OF COOL-CLIMATE RIESLING

Abstract

Yeast assimilable nitrogen (YAN) is regularly supplemented during commercial winemaking, but excessive YAN supplementation may lead to negative sensory outcomes, particularly when using Diammonium Phosphate (DAP). To define both the optimal level and ideal type of YAN supplementation for cool-climate Riesling fermentations, juice was supplemented with one of four nutrient types, comparing inorganic (ammonia, or AMM) and organic (Primary Amino Nitrogen, or PAN) nitrogen at two supplementation levels. To prevent stuck or sluggish fermentation, This study examined two supplementation levels, the first corresponds to the level generally considered to be the minimum YAN required for a successful fermentation (150ppm N/L) and the second with the level that has been cited as more appropriate to avoid stuck or sluggish fermentation (250ppm N/L). Of the four nutrient treatments, two nutrient types consisted of primarily inorganic nitrogen, and they had generally lower pH, higher Titratable Acidity (TA), and lower acetic acid, whereas fermentations supplemented with the two nitrogen sources consisting of primarily organic nitrogen showed increased fermentation kinetics and enhanced the ability to achieve dryness (<2g/L residual sugar). A panel of wine experts analyzed the sensory properties of the wines, using a projective mapping technique known as Napping®. Sensory characteristics such as 'mineral', 'petrol', and 'balanced' were most closely associated with lower YAN supplementation levels and some organic nutrient types.

Introduction

During the course of grape juice fermentation into wine, yeast nutrition is key to maintaining optimal yeast biomass and vitality. Yeast Assimilable Nitrogen (YAN) comprises those nitrogenous compounds that can be metabolized by *S. cerevisiae*, and is calculated as the sum of the concentrations of ammonia

(AMM) and primary amino nitrogen (PAN). The nitrogenous composition of grape musts vary widely, consisting of differing mixtures of ammonium, protein, peptides, and amino acids. The resultant quantitative and qualitative variation of the grape must nitrogen content can affect both the kinetics of fermentation as well as the aromatic composition of the final wine (Amparo and Fleet 2006). Riesling, in particular, retains a higher proportion of total amino nitrogen in berry skins, which are generally discarded during processing. For this reason, up to 30% of Riesling YAN will be lost prior to fermentation, potentially increasing reliance on nitrogen supplementation (Stines et al. 2000).

YAN supplementation as part of commercial winemaking is a common practice, particularly in regions with consistently low must YAN. A lack of YAN can result in a stuck or sluggish fermentation from either lack of maximal yeast biomass or insufficient nutrient supply throughout the fermentation (Bisson and Butzke 2000). Insuffucient YAN for yeast metabolism often results in the alteration of metabolic pathways of the Sulfate Reduction Sequence (SRS), causing the release of hydrogen sulfide (H_2S), which is known to cause an unpleasant aroma with very low sensory thresholds (Bell and Henschke 2005). The reported minimum YAN concentration required to complete fermentation is 140 mg N/L (Dukes and Butzke 1998), but recommendations rise upwards of 250 mg N/L based on enological parameters such as yeast strain, sugar content, and targeted fermentation time or aromatic composition. (Bisson and Butzke 2000).

YAN supplementation during fermentation has shown to impact the production of volatile compounds including fatty acids, fusel alcohols, and acetate and ethyl esters (Bell and Henschke 2005, Torrea et al. 2011, Vilanova et al. 2012). Inorganic nitrogen is most commonly added to grape musts, typically in the form of diammonium phosphate (DAP). While inorganic ammonium is the preferred source for biomass formation, amino acids are preferentially used during the stationary phase for cell maintenance, influencing volatile composition (Beltran et al. 2005). Wines supplemented with organic nitrogen or a combination of ammonium and organic nitrogen have been shown to have a higher concentration of

acetate and ethyl esters, compared to wines supplemented solely with DAP (Torrea et al. 2011, Barbosa et al. 2012).

Previous studies that compared the volatile profiles of inorganic and organic nitrogen sources were generally performed in synthetic media (Jiranek and Henschke 1995, Vilanova et al. 2007, Barbosa, et al. 2012), which informs volatile quantification but precludes the types of sensory evaluation necessary for commercial applications. Additionally, one study comparing inorganic versus organic nitrogen sensory impacts in wine utilized YAN levels of 320-480 mg N/L (Torrea et al. 2011), a supplementation level not achievable in musts as deficient as cool climate Riesling, given current legal addition rates of available nutrients. Lastly, recent work suggested that as additions of DAP increased, the wines were less preferred by sensory panelists (Tahim et al., unpublished). Given the historically low YAN of Riesling, widespread YAN additions in the cellar, and the potentially negative outcomes of large amounts of nutrient addition, this study sought to determine the optimal YAN addition level and nutrient type to maximize fermentation performance and sensory outcomes in YAN deficient cool climate Riesling.

Materials & Methods

Yeast Selection. All treatments were inoculated with either *Saccharomyces bayanus* yeast strain EC1118 (*Lallemand*) or *Saccharomyces cerevisiae* strain W15 (*Lallemand*). These strains were chosen due to their popularity in the Finger Lakes for Riesling fermentation as well as their differing nitrogen requirements, as noted by the manufacturer.

Nitrogen Supplementation. Concentrations of YAN, calculated as the sum of primary amino nitrogen (PAN) and ammonia (AMM), were supplemented at two levels: 150ppm N/L, near the estimated minimum YAN for successful fermentation (Bell and Henschke 2005), and 250ppm N/L, consistent with recommendations to avoid a stuck or sluggish fermentation (Bisson and Butzke 2000). Both supplementation levels (150ppm and 250ppm) were realized using one of four nitrogen supplementation treatments. The first treatment (T1), the control, was comprised solely of inorganic nitrogen in the form

of diammonium phosphate (DAP). The second treatment (T2) contained both organic and inorganic nitrogen sources. Organic nitrogen was added in the form of Fermaid O (*Lallemand*), supplemented up to the manufacturer's recommended dosage of 400mg/L, which contributes 16mg/L YAN; DAP was added to achieve YAN levels of 150ppm and 250ppm. The third treatment (T3) was supplemented with a proportional amino acid mixture like that found in Riesling (Spayd and Andersen-Bagge 1996); food-grade amino acids (Sigma Aldrich, St. Louis, MO, USA) were added to achieve desired N concentrations. The fourth treatment (T4) was prepared using only Fermaid O (*Lallemand*), in excess of the manufacturer's recommendation of 400mg/L, at 4.07g/L Fermaid O. All treatments at the two supplementation levels were performed in duplicate.

Model Juice Preparation. Chemically defined grape juice medium (CDGJM), adapted from Wang et al. 2003, and modified from 120g/L to 100g/L of glucose and fructose, was used for all model juice fermentations. Assembly of the CDGM was perfomed in 2L Erlenmeyer flasks, using reagents from Sigma Aldrich (St. Louis, MO) and Thermo Fischer Scientific (Waltham, MA), and the model juice was then aliquoted into 250mL No.8 Erlemenyer flasks.

Model Juice Fermentations. Yeast was rehydrated using the manufacturer's protocol, by dissolving 0.3g/L GoFerm (Scott Laboratories, Petaluma, CA, USA) in deionized water at 40° C, contributing 10mg/L of nitrogen to the CDGJM YAN. Yeast was then added to the CDGJM at a rate of 0.4g/L. CDGJM fermentations took place in the 250mL, No.8 Erlenmeyer flasks fitted with three-piece airlocks, and placed in a temperature-controlled orbital shaker set to 25 rpm and maintained at 18° C. Once fermentations ceased CO_2 production, residual sugar (RS) was estimated using CliniTest Tablets (Bayer Inc., Elkhart IN, USA), and final samples of the model wine were taken.

Fruit Processing. Riesling grapes were obtained from Cornell University's Lansing Research Vineyards in the Finger Lakes of New York during the 2015 harvest season. Harvesting took place on October 9th, once vineyard sampling showed the fruit had reached 20° Brix. A total of 1,182.5kg of fruit were hand

harvested to exclude clusters infected with 10% or more with *Botrytis cinerea*, collected into picking bins, and transported to the Cornell Orchards winery. Bins were emptied into a destemmer (Bucher Delta E1, Rivesaltes, Switzerland) and pumped into a membrane press (Scharfenberger Tx3 Europress, Bad Dürkheim, Germany). The fruit was divided into 4 press loads, which were pressed in an increasing pressure stage program up to a maximum of 1.7 bars for a total of 140 minutes per press load. An addition of 50mg/L of sulfur dioxide (SO_2) was introduced into the crush pan during each press iteration in the form of potassium metabisulfite ($K_2S_2O_5$) in order to prevent oxidation of the juice. Juice was collected into a 750L plastic tank, mixed to homogenize, and was allowed to settle overnight at 7.2° C. The following day, 11.3L aliquots of juice were racked off the gross lees into thirty-two 19L carboys fitted with three-piece airlocks.

Riesling Fermentations. Active dry yeast were rehydrated and inoculated using the manufacturer's protocol, by dissolving 0.3g/L GoFerm (Scott Laboratories, Petaluma, CA, USA) in deionized water at 40° C, contributing 10mg/L of nitrogen to the juice YAN. Yeast was then added to the juice at a rate of 0.4g/L. Fermentations took place in a temperature-controlled cold-room maintained at 18° C. Once CO_2 production ceased, residual sugar (RS) was estimated using CliniTest Tablets (Bayer Inc., Elkhart IN, USA), and wines were racked off lees into 11.3L carboys when less than 1% sugar remained. Sixty mg/L of SO_2 was added to the wine, which was placed in cold storage at 4° C. Temperature was adjusted to 2° C for 30 days in order to tartrate stabilize the wines, after which wine was racked off the fine lees and tartrates and returned to 4° C storage until bottling. Prior to bottling, 10mL samples were taken for analysis of titratable acidity. Bottles were then filled manually in 750mL bottles (Waterloo Container Company, Waterloo, NY, USA), and screw-capped using a bottling line with screw-cap attachment (Prospero GAI 1006, Ceresole d'Alba, Italy).

Sampling. For each fermentation, duplicate 2mL samples were taken daily from each flask or carboy using 5mL and 25mL disposable sterile pipettes (Celltreat, Shirley, MA, USA) for model juice and

Riesling fermentations, respectively. Samples were stored in 2mL Eppendorf tubes at -15° C until analyzed.

Analytical Methods. The concentrations of sugars (glucose and fructose) and organic acids (tartaric, malic, citric, lactic, and acetic) were quantified using high performance liquid chromatography (HPLC) on an Agilent Systems 1100 HPLC (Agilent Technologies, Santa Clara, CA, USA), equipped with photodiode array detector and refractive index on Cation Exchange Column (Bio-Rad Aminex HPX-87H, 300mm x 7.8mm). The analysis method was isocratic at 0.5mL/min, using 6% acetonitrile in 0.045N sulfuric acid mobile phase and a column temperature of 45° C. YAN was calculated using a Chemwell 2910 Multianalyzer (Unitech Scientific, Hawaiian Gardens, CA, USA) to perform separate enzymatic tests of primary amino nitrogen (PAN) and ammonia (AMM). AMM was quantified with a glutamate dehydrogenase enzymatic assay (Ough 1969), using an enzymatic kit (Unitech Scientific, Ammonia Extended Range UniTAB, 2007). PAN was determined by a N-acetylcysteine/ o-phthaldehdye spectrophotometric assay (NOPA) (Dukes and Butzke 1998), using an enzymatic kit (Unitech Scientific, Primary Amino Nitrogen UniTAB, 2007).

Sensory Evaluation. Wines were evaluated using a modified projective mapping technique, known as Napping®, using a panel of 17 wine professionals, including winemakers and extension personnel. The panel consisted of 7 males and 10 females, aged 21-66 years. Panelists convened at the tasting room of Fox Run Vineyards (Penn Yan, NY). Fifty mL wine samples were served in matching 300mL ISO tasting glasses labeled with three-digit random numbers, covered with petri dishes, and presented to panelists in balanced order dictated by Williams Latin Square. Water, spit cups, paper napkins, and unsalted crackers were provided to the panelists. The wines were divided by yeast strain into two flights of 12 wines. Each flight contained one fermentation replicate from each of the eight treatments and four internal controls- two from fermentation replicates and two sample replicates, identical to another sample in the flight.

The panelists executed a Napping® Test (Pagès 2005) on a 60cmx40cm white butcher paper, where they were instructed to place similar samples physically close together and different samples were placed physically far apart. The criteria for similarity was self-derived for each panelist, according to the methods outlined in Pagès et al. 2005.

Statistical Analysis. Statistical analysis of wine chemical parameters and sensory evaluation was performed using R Studio version 3.3.3 (R Foundation for Statistical Computing, Vienna, Austria). Wine chemistry parameters were analyzed using a linear model, predicting means based on a full factorial design of four treatments by two levels by two strains, creating a 4x2x2 ANOVA. Post-hoc analyses and estimated predicted means were calculated with the R package lsmeans (Lenth 2016).

Analysis of the Napping® sensory data was performed using the R packages SensoMineR (Lê and Husson 2008) and FactoMineR (Lê, Josse and Husson 2008) computing environments. The Euclidean distances of the wine samples were compiled into a consensus configuration and analyzed using Multi-Factor Analysis (MFA) (Morand and Pagès 2006). The consensus configuration was derived by performing a PCA on the panelists' arrangement of the individual wine samples by X and Y coordinates on the nappe, which were then normalized among panelists by dividing all of the components by the first eigenvalue. A second PCA was then performed on the normalized data to generate the consensus map, and a Procrustes rotation, based on the RV coefficient's statistical measure of fit between configurations, then allowed for maximum agreement between panelists' configurations. The final location on the PCA plot represents the Procrustes rotation of the consensus map. The descriptors were compiled into attribute counts and treated as supplementary variables. The supplementary variables were represented as a PCA model based on their correlation coefficient with each axis of the sample loadings (Perrin et al. 2008). Only statistically significant attribute counts were utilized in the PCA model.

Results

Initial Model Juice Chemistry. The initial chemistry for the CDGJM was pH 3.27, titratable acidity (TA) 4.78g/L expressed as tartaric acid equivalents (TAE), 44mg/L YAN, and 200g/L sugars (glucose + fructose).

Effects of Nutrient Type and Supplementation Level on Model Wine Chemistry. The pH of the model wines was affected by nutrient treatment; pH was higher when supplemented with T4 (Table 1.1), and yeast strain altered final wine pH ($p<0.01$). Model wines supplemented to Y2 had lower TA for all nutrient types than those supplemented to Y1 (Table 1.2), and yeast strain had an effect on final titratable acidity ($p<0.01$). Acetic acid production was not affected by strain, supplementation level, or nutrient type (Table 1.4). Changes in final tartaric acid were not clearly associated with strain choice, nutrient type, or nutrient level (Table 1.5), and ethanol values were only independently affected by yeast strain ($p<0.01$).

Table 1.1 Mean pH in model wines produced with two yeast strains and two yeast assimilable nitrogen (YAN) must levels using four YAN supplementation treatments (T1-T4).

Yeast Strain	EC1118		W15	
YAN	150ppm	250ppm	150ppm	250ppm
T1	3.35^{b}	3.38^{b}	3.28^{b}	3.27^{b}
T2	3.38^{b}	3.34^{b}	3.29^{b}	3.28^{b}
T3	3.14^{a}	3.15^{a}	3.15^{a}	3.17^{a}
T4	3.49^{c}	3.56^{d}	3.38^{c}	3.51^{d}

T1 = Diammonium Phosphate (DAP), T2 = DAP+ Fermaid O, T3 = amino acids, T4= Fermaid O
Within a yeast strain, means followed by different letters indicate significant difference from each other using Tukey's test (p<0.05).

Table 1.2 Mean titratable acidity[1] (g/L) in model wines produced with two yeast strains and two yeast assimilable nitrogen (YAN) must levels using four YAN supplementation treatments (T1-T4).

Yeast Strain	EC1118		W15	
YAN	150ppm	250ppm	150ppm	250ppm
T1	8.47^{a}	7.29^{a}	10.16^{cd}	9.84^{cd}
T2	8.58^{a}	7.26^{a}	10.92^{d}	8.68^{abc}
T3	7.32^{a}	6.99^{a}	7.64^{ab}	6.98^{a}
T4	7.74^{a}	7.27^{a}	9.22^{bcd}	7.23^{ab}

[1]Expressed as Tartaric Acid Equivalents (TAE)
T1 = Diammonium Phosphate (DAP), T2 = DAP+ Fermaid O, T3 = amino acids, T4= Fermaid O
Within a yeast strain, means followed by different letters indicate significant difference from each other using Tukey's test (p<0.05).

Table 1.3 Mean ethanol (%v/v) in model wines produced with two yeast strains and two yeast assimilable nitrogen (YAN) must levels using four YAN supplementation treatments (T1-T4).

Yeast Strain	EC1118		W15	
YAN	150ppm	250ppm	150ppm	250ppm
T1	10.61^{bc}	10.57^{bc}	9.78^{ab}	10.06^{ab}
T2	10.57^{c}	10.62^{abc}	10.06^{a}	9.67^{ab}
T3	10.62^{ab}	10.46^{a}	9.67^{b}	10.03^{ab}
T4	10.46^{abc}	10.11^{abc}	10.03^{ab}	10.21^{ab}

T1 = Diammonium Phosphate (DAP), T2 = DAP+ Fermaid O, T3 = amino acids, T4= Fermaid O
Within a yeast strain, means followed by different letters indicate significant difference from each other using Tukey's test (p<0.05).

Table 1.4 Mean acetic acid (g/L) in model wines produced with two yeast strains and two yeast assimilable nitrogen (YAN) must levels using four YAN supplementation treatments (T1-T4).

Yeast Strain	EC1118		W15	
YAN	150ppm	250ppm	150ppm	250ppm
T1	0.63^{a}	0.67^{a}	0.63^{a}	0.53^{a}
T2	0.67^{a}	0.62^{a}	0.53^{a}	0.55^{a}
T3	0.62^{a}	0.63^{a}	0.55^{a}	0.49^{a}
T4	0.63^{a}	0.46^{a}	0.49^{a}	0.73^{a}

T1 = Diammonium Phosphate (DAP), T2 = DAP+ Fermaid O, T3 = amino acids, T4= Fermaid O
Within a yeast strain, means followed by different letters indicate significant difference from each other using Tukey's test (p<0.05).

Table 1.5 Mean tartartic acid (g/L) in model wines produced with two yeast strains and two yeast assimilable nitrogen (YAN) must levels using four YAN supplementation treatments (T1-T4).

Yeast Strain	EC1118		W15	
YAN	150ppm	250ppm	150ppm	250ppm
T1	1.20^{a}	1.51^{ab}	2.02^{ab}	1.88^{ab}
T2	1.06^{b}	1.65^{ab}	1.93^{ab}	1.68^{ab}
T3	3.35^{ab}	3.13^{ab}	2.19^{ab}	3.65^{b}
T4	1.00^{a}	1.34^{ab}	1.69^{ab}	1.22^{a}

T1 = Diammonium Phosphate (DAP), T2 = DAP+ Fermaid O, T3 = amino acids, T4= Fermaid O
Within a yeast strain, means followed by different letters indicate significant difference from each other using Tukey's test (p<0.05).

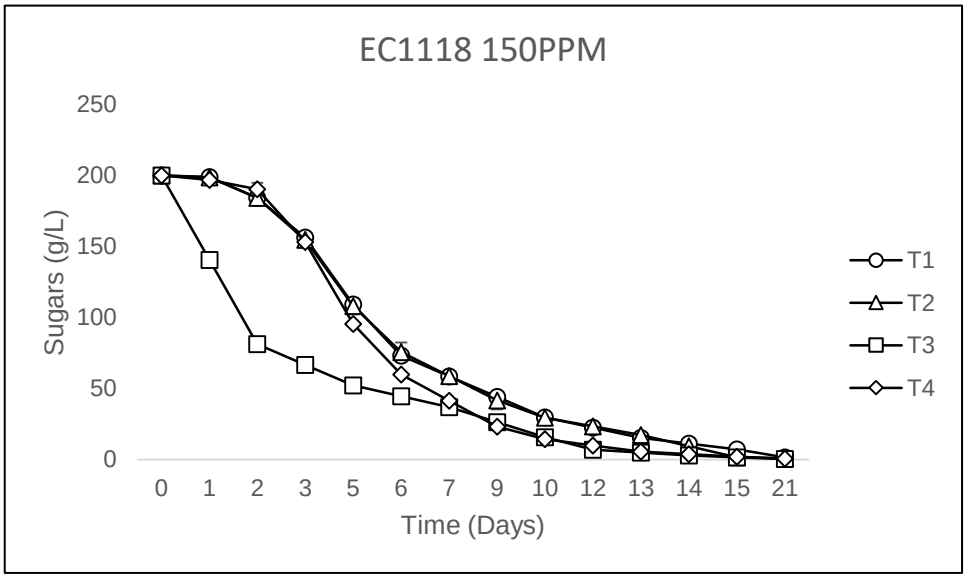
Effects of Nutrient Type and Supplementation Level on Model Wine Fermentation Kinetics.

At 150ppm YAN, T3 showed the fastest sugar consumption for both strains (Figure 1.1). In EC1118, T3 and T4 at 150ppm reached final sugar depletion on the same time (Day 13), but in W15, T3 sugar was depleted before T4 (Figure 1.1).

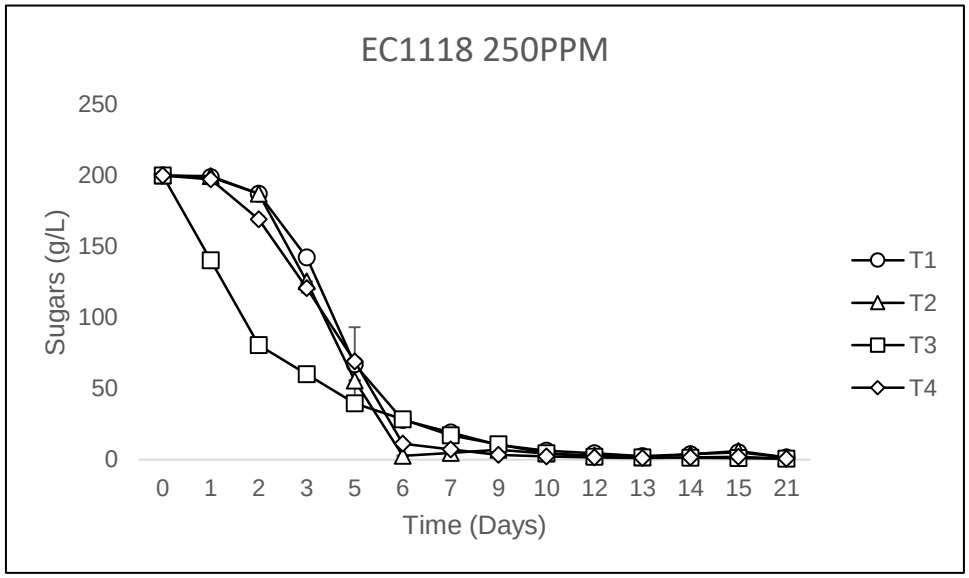
At the 250ppm supplementation level T2, followed closely by T4, resulted in the most rapid sugar depletion with EC1118; T4 was fastest with W15. For both strains at both supplementation levels,

treatments containing organic nutrients (T2-T4) had faster sugar consumption kinetics than the DAP-only treatment, T1 (Figure 1.1).

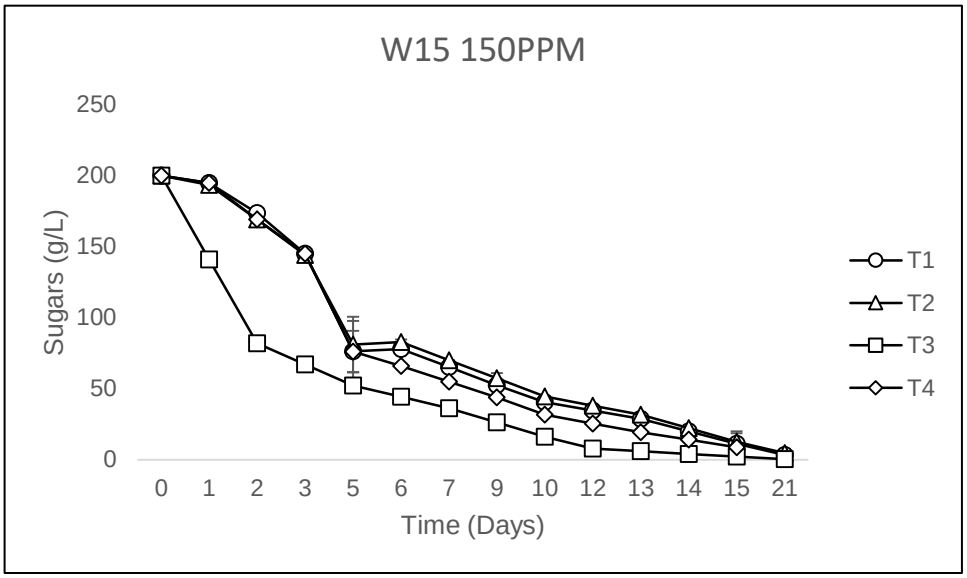
A)



B)



C)



D)

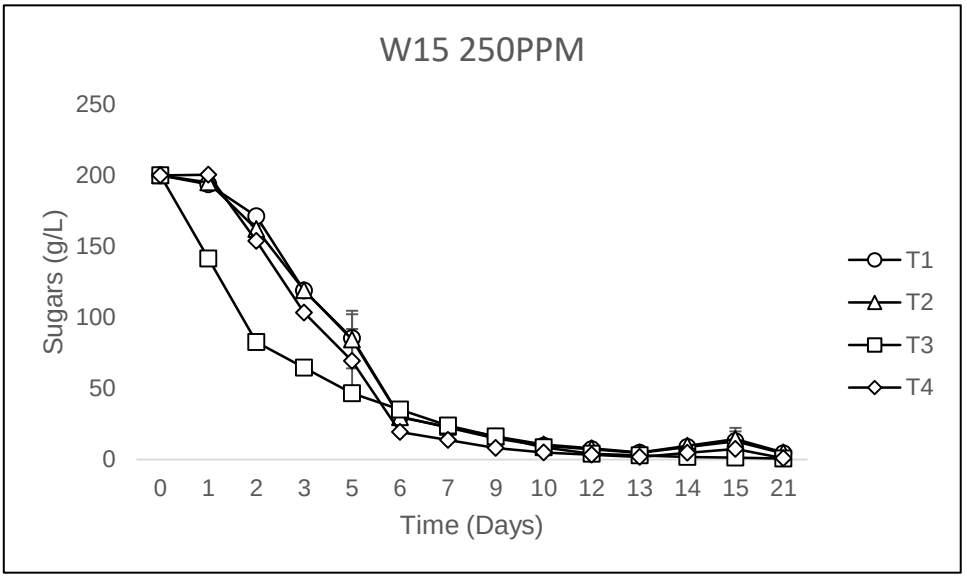
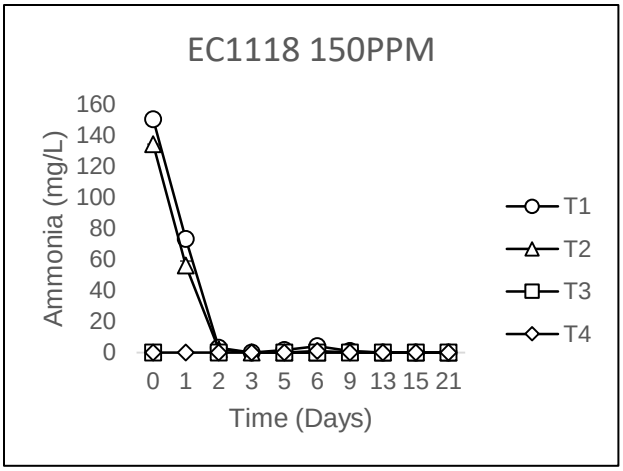


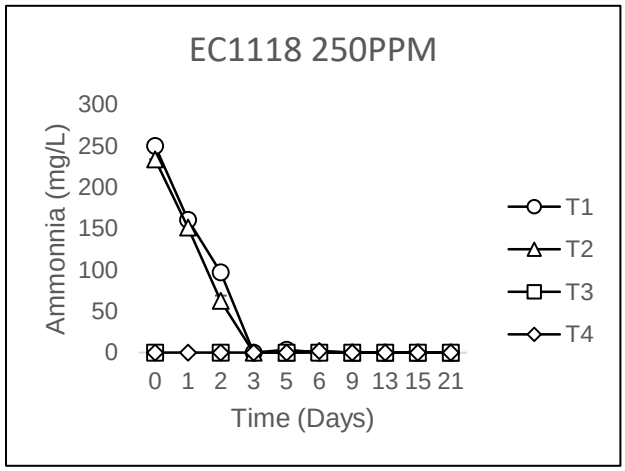
Figure 1.1 Sugar (glucose +fructose) consumption during fermentation of model wine by EC1118 (A, B) and W15 (C, D) at two nitrogen supplementation levels and four supplementation treatments(T1-T4). Error bars represent Standard Error between fermentation replicates. *T1 = DAP, T2 = DAP +Fermaid O, T3 = amino acids, T4= Fermaid O.*

Model Wine YAN Consumption. Nitrogen in T3 and T4 consisted primarily of PAN. Ammonia depletion occurred in both strains by Day 2 for 150ppm and Day 3 for 250ppm (Figure 1.2). PAN was also depleted by Day 2 at 150ppm for both strains. At 250ppm, PAN was depleted by Day 5 for EC1118 and Day 3 for W15 (Figure 1.2).

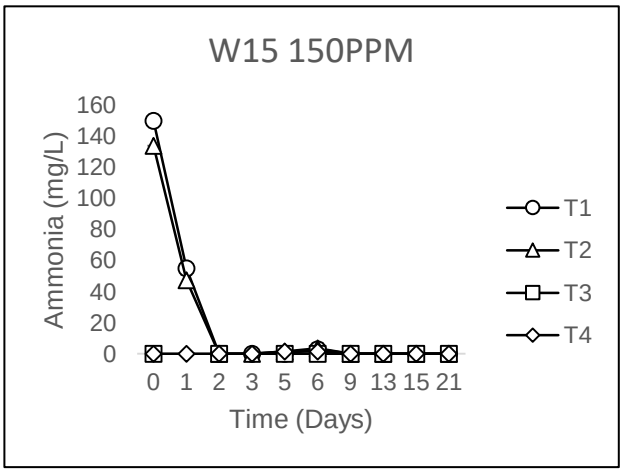
A)


EC1118 150PPM
Ammonia (mg/L)
160
140
120
100
80
60
40
20
0
0 1 2 3 5 6 9 13 15 21
Time (Days)
T1
T2
T3
T4

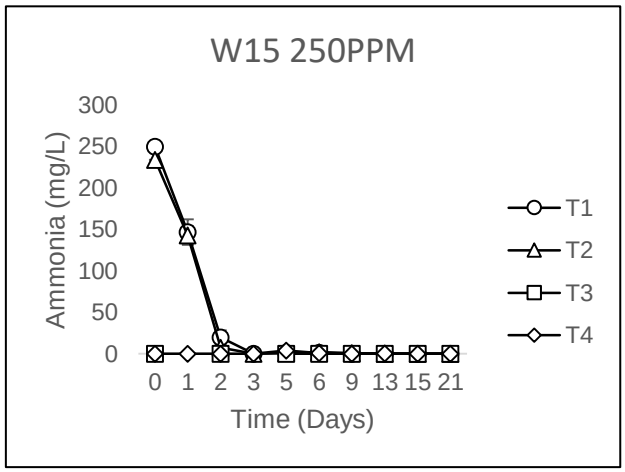
B)


EC1118 250PPM
Ammonnia (mg/L)
300
250
200
150
100
50
0
0 1 2 3 5 6 9 13 15 21
Time (Days)
T1
T2
T3
T4

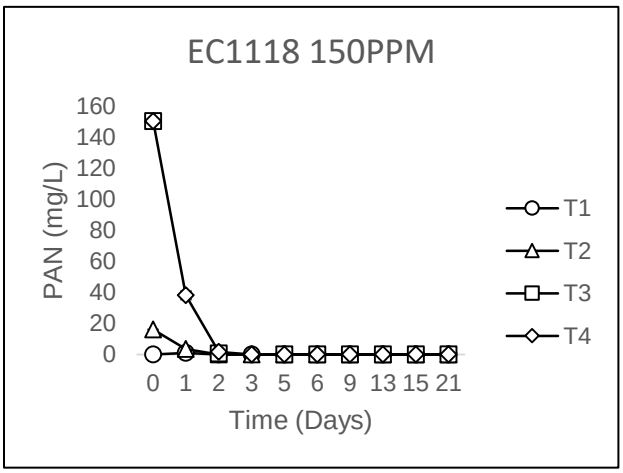
C)


W15 150PPM
Ammonia (mg/L)
160
140
120
100
80
60
40
20
0
0 1 2 3 5 6 9 13 15 21
Time (Days)
T1
T2
T3
T4

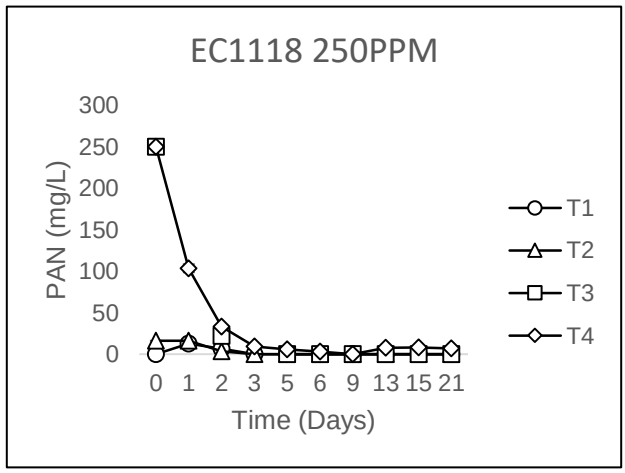
D)


W15 250PPM
Ammonia (mg/L)
300
250
200
150
100
50
0
0 1 2 3 5 6 9 13 15 21
Time (Days)
T1
T2
T3
T4

E)


EC1118 150PPM
PAN (mg/L)
160
140
120
100
80
60
40
20
0
0 1 2 3 5 6 9 13 15 21
Time (Days)
T1
T2
T3
T4

F)


EC1118 250PPM
PAN (mg/L)
300
250
200
150
100
50
0
0 1 2 3 5 6 9 13 15 21
Time (Days)
T1
T2
T3
T4

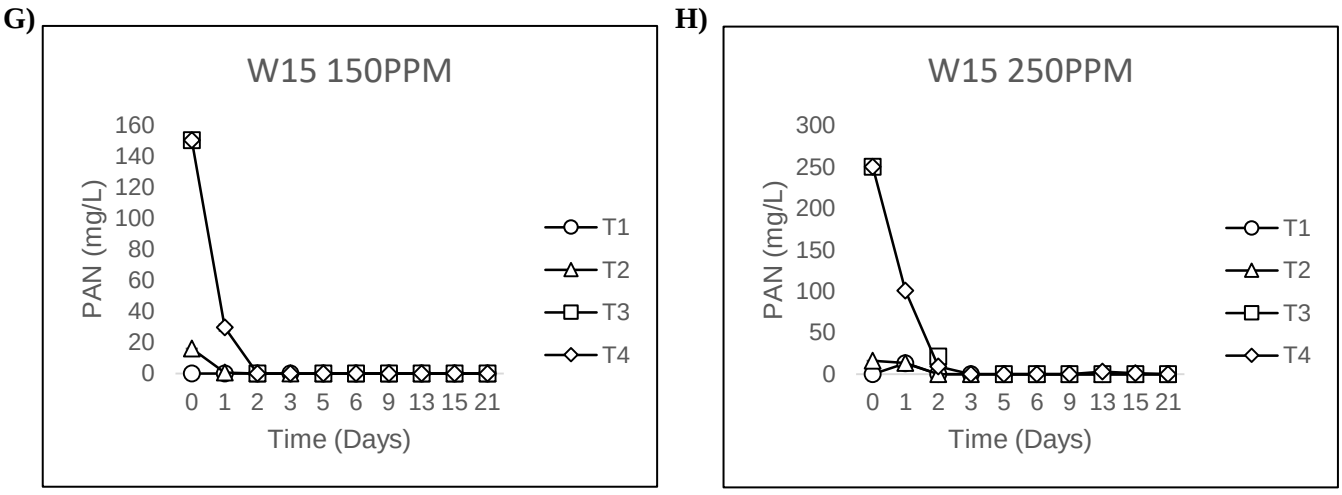


Figure 1.2 Ammonia (A-D) Primary Amino Nitrogen (E-H) consumption during fermentation of model wine by EC1118 (A, B, E, F) and W15 (C, D, G, H). Wines underwent four different nutrient treatments (T1-T4) at two different supplement levels. Error bars represent Standard Error between duplicate fermentations. *T1 = DAP, T2 = DAP +Fermaid O, T3 = amino acids, T4= Fermaid O.*

Initial Riesling Juice Chemistry. Prior to fermentation, Riesling juice was measured at 19.6° Brix, 7.8g/L TA in TAE, and pH 3.3 ± 0.01. The initial juice YAN was 39mg/L PAN and 38mg/L AMM, such that total YAN was 77mg/L.

Effects of Nutrient Type and Supplementation Level on Riesling Wine Chemistry. The final wine chemistry of the fermentations varied by yeast strain, nutrient type and supplementation level. In EC1118, there were differences among all treatments for pH (Table 1.6), with inorganic nitrogen treatments (T1 and T2) resulting in lower pH than organic nitrogen (T3 and T4). At the higher supplementation level (250ppm) these effects on pH were greater. Titratable acidity did not vary among the four nutrient types at 150ppm, but at 250ppm T1 and T2 had higher TA than T3 and T4. Trends were similar in W15 fermentations, except that TA was lower for all treatments at the lower supplementation level (Table 1.7). Acetic acid production was affected by strain, supplementation level, and most nutrient types (Table 1.9). Inorganic treatments (T1 and T2) had lower acetic acid concentrations, and were undetectable in EC1118 at the higher supplementation rate (Table 1.9). Malic acid content in the final wine also differed by strain, and W15 had lower final malic acid at the higher supplementation rate (Table 1.11).

Table 1.6 Mean pH in Riesling wines produced with two yeast strains and two yeast assimilable nitrogen (YAN) must levels using four YAN supplementation treatments (T1-T4).

Yeast Strain	EC1118		W15	
YAN	150ppm	250ppm	150ppm	250ppm
T1	3.21^{a}	3.18^{a}	3.14^{a}	3.12^{a}
T2	3.27^{b}	3.22^{a}	3.13^{a}	3.15^{a}
T3	3.33^{c}	3.37^{cd}	3.22^{b}	3.27^{c}
T4	3.38^{d}	3.45^{e}	3.26^{bc}	3.34^{d}

T1 = Diammonium Phosphate (DAP), T2 = DAP+ Fermaid O, T3 = amino acids, T4= Fermaid O
Within a yeast strain, means followed by different letters indicate significant difference from each other using Tukey's test (p<0.05).

Table 1.7 Mean titratable acidity[1] (g/L) in Riesling wines produced with two yeast strains and two yeast assimilable nitrogen (YAN) must levels using four YAN supplementation treatments (T1-T4).

Yeast Strain	EC1118		W15	
YAN	150ppm	250ppm	150ppm	250ppm
T1	6.35^{b}	6.47^{b}	7.83^{d}	7.46^{bcd}
T2	6.20^{ab}	6.43^{b}	7.66^{cd}	7.24^{bc}
T3	6.14^{ab}	5.66^{a}	6.97^{ab}	6.43^{a}
T4	6.10^{ab}	5.98^{ab}	6.61^{a}	6.44^{a}

[1]Expressed as Tartaric Acid Equivalents (TAE)
T1 = Diammonium Phosphate (DAP), T2 = DAP+ Fermaid O, T3 = amino acids, T4= Fermaid O
Within a yeast strain, means followed by different letters indicate significant difference from each other using Tukey's test (p<0.05).

Table 1.8 Mean ethanol (%v/v) in Riesling wines produced with two yeast strains and two yeast assimilable nitrogen (YAN) must levels using four YAN supplementation treatments (T1-T4).

Yeast Strain	EC1118		W15	
YAN	150ppm	250ppm	150ppm	250ppm
T1	11.63^{a}	11.39^{a}	11.46^{a}	11.59^{a}
T2	11.35^{a}	11.32^{a}	11.48^{a}	11.47^{a}
T3	11.48^{a}	11.33^{a}	11.47^{a}	11.44^{a}
T4	11.70^{a}	11.25^{a}	11.55^{a}	11.39^{a}

T1 = Diammonium Phosphate (DAP), T2 = DAP+ Fermaid O, T3 = amino acids, T4= Fermaid O
Within a yeast strain, means followed by different letters indicate significant difference from each other using Tukey's test (p<0.05).

Table 1.9 Mean acetic acid (g/L) in Riesling wines produced with two yeast strains and two yeast assimilable nitrogen (YAN) must levels using four YAN supplementation treatments (T1-T4).

Yeast Strain	EC1118		W15	
YAN	150ppm	250ppm	150ppm	250ppm
T1	0.29^{bc}	0.00^{a}	0.34^{ab}	0.18^{a}
T2	0.35^{bc}	0.14^{ab}	0.38^{ab}	0.34^{ab}
T3	0.36^{bc}	0.27^{abc}	0.44^{ab}	0.44^{ab}
T4	0.47^{c}	0.43^{c}	0.56^{ab}	0.60^{b}

T1 = Diammonium Phosphate (DAP), T2 = DAP+ Fermaid O, T3 = amino acids, T4= Fermaid O
Within a yeast strain, means followed by different letters indicate significant difference from each other using Tukey's test (p<0.05).

Table 1.10 Mean tartaric acid (g/L) in Riesling wines produced with two yeast strains and two yeast assimilable nitrogen (YAN) must levels using four YAN supplementation treatments (T1-T4).

Yeast Strain	EC1118		W15	
YAN	150ppm	250ppm	150ppm	250ppm
T1	3.19^{d}	3.16^{cd}	3.10^{b}	3.16^{b}
T2	3.13^{bcd}	3.19^{d}	3.19^{b}	3.10^{b}
T3	3.00^{ab}	3.03^{abc}	3.11^{b}	3.08^{ab}
T4	2.95^{a}	2.87^{a}	3.07^{ab}	2.93^{a}

T1 = Diammonium Phosphate (DAP), T2 = DAP+ Fermaid O, T3 = amino acids, T4= Fermaid O
Within a yeast strain, means followed by different letters indicate significant difference from each other using Tukey's test (p<0.05).

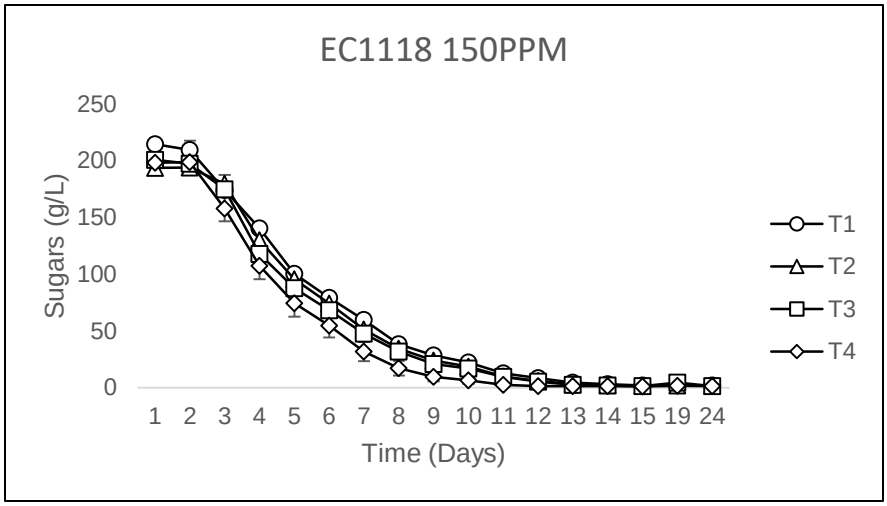
Table 1.11 Mean malic acid (g/L) in Riesling wines produced with two yeast strains and two yeast assimilable nitrogen (YAN) must levels using four YAN supplementation treatments (T1-T4).

Yeast Strain	EC1118		W15	
YAN	150ppm	250ppm	150ppm	250ppm
T1	2.63^{ab}	2.53^{a}	3.39^{c}	2.96^{a}
T2	2.63^{ab}	2.63^{ab}	3.44^{c}	2.95^{a}
T3	2.54^{a}	2.54^{a}	3.32^{bc}	3.15^{b}
T4	2.66^{ab}	2.78^{b}	3.36^{c}	3.28^{bc}

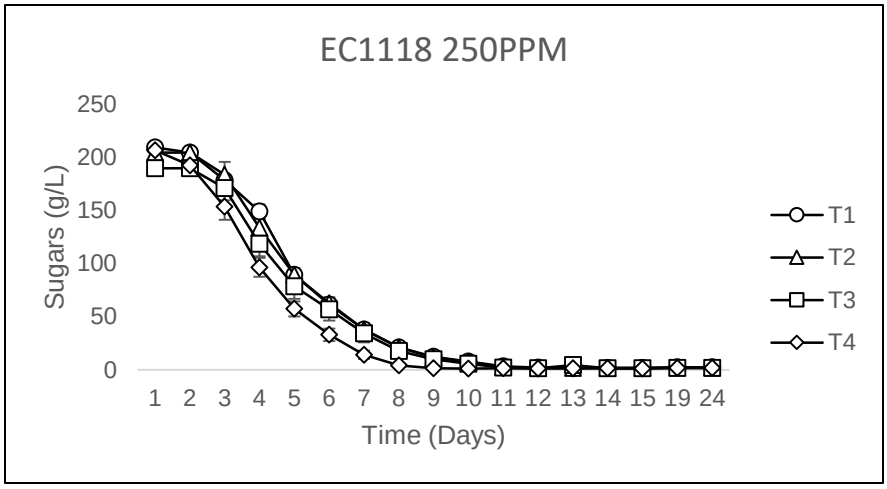
T1 = Diammonium Phosphate (DAP), T2 = DAP+ Fermaid O, T3 = amino acids, T4= Fermaid O
Within a yeast strain, means followed by different letters indicate significant difference from each other using Tukey's test (p<0.05).

Effects of Nutrient Type and Supplementation Level on Riesling Fermentation Kinetics. Length of fermentation was influenced by a three-way interaction between yeast strain, nutrient type, and supplementation level, meaning that all had a significant effect on fermentation kinetics (p=0.007). EC1118 was a faster fermenter than W15 for all nutrient types and supplementation levels, and the higher supplementation level resulted in faster fermentation completion for all nutrient treatments (Figure 1.3). Among nutrient types, Treatments T3 and T4 completed fermentation more quickly than the DAP containing treatments, T1 and T2, and T4 resulted in noticeably faster fermentation time than other treatments at both 150ppm and 250ppm. For strain W15, both nutrients containing organic nitrogen (T2-T4) reached completion faster than T1 (Figure 1.3). Similarly, T4 had more rapid sugar consumption than any other treatment (Figure 1.3).

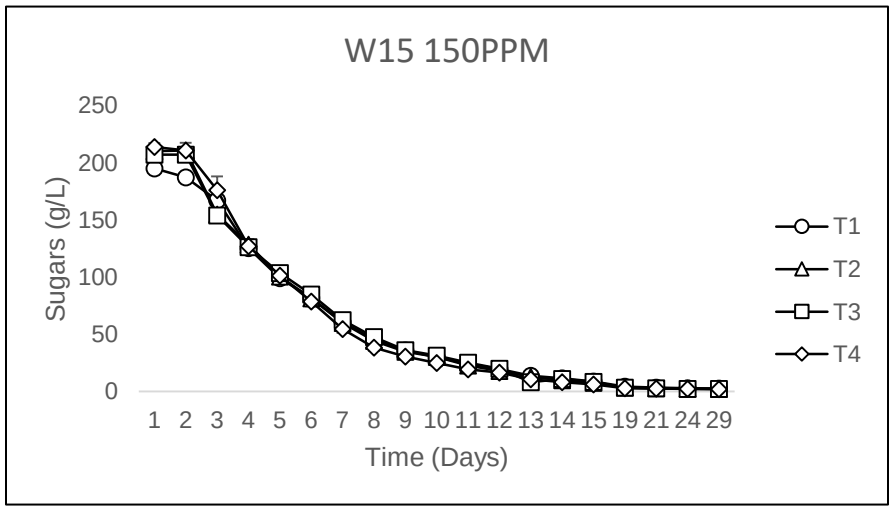
A)


EC1118 150PPM
Sugars (g/L)
250
200
150
100
50
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 19 24
Time (Days)
T1
T2
T3
T4

B)


EC1118 250PPM
Sugars (g/L)
250
200
150
100
50
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 19 24
Time (Days)
T1
T2
T3
T4

C)


W15 150PPM
Sugars (g/L)
250
200
150
100
50
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 19 21 24 29
Time (Days)
T1
T2
T3
T4

D)

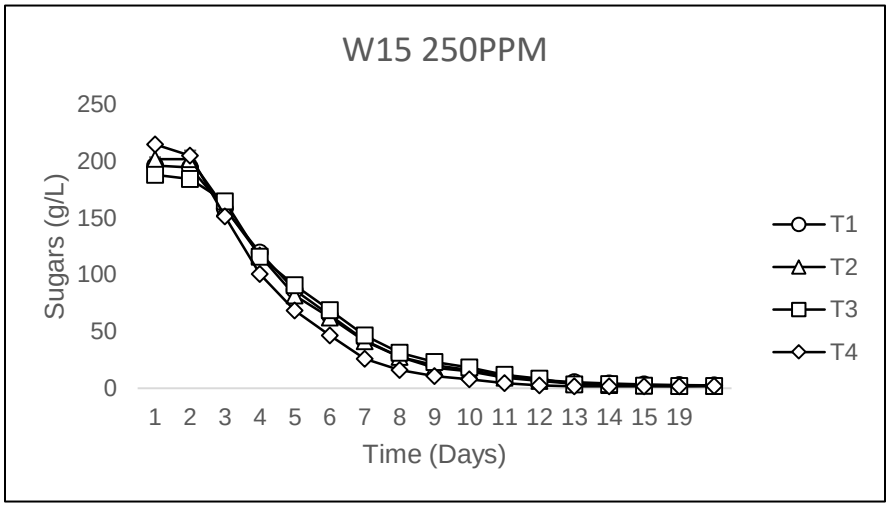


Figure 1.3. Four nutrient treatments (T1-T4) at two supplementation levels, 150ppm (A & C) and 250ppm (B&D). Error bars represent Standard Error between two fermentation replicates. *T1 = DAP, T2 = DAP +Fermaid O, T3 = amino acids, T4= Fermaid O.*

All nutrient types and supplementation levels achieved dryness (<2g/L) with EC1118, yet only Fermaid O (T4) fermentations reached dryness with W15. Increasing supplementation from 150ppm to 250ppm did not necessarily decrease the final amount of residual sugar (Table 1.12).

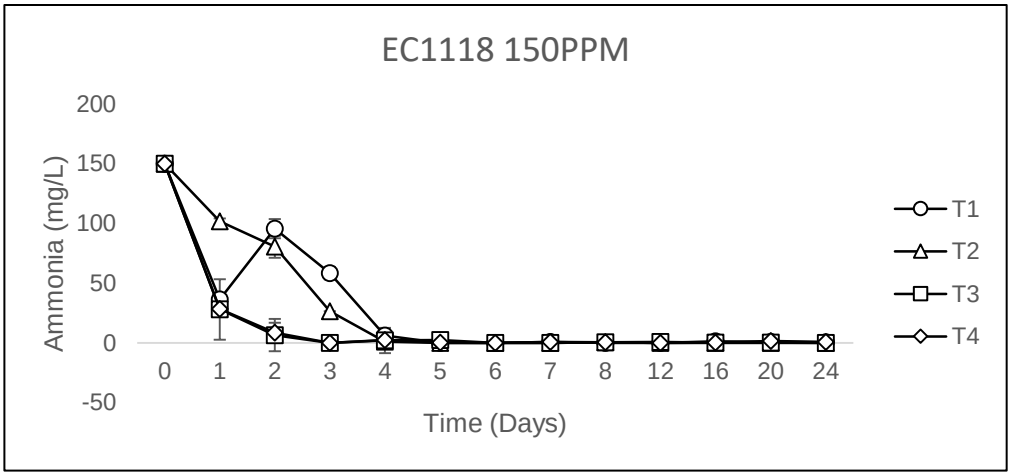
Table 1.12. Mean residual sugar (g/L) in Riesling wines produced with two yeast strains and two yeast assimilable nitrogen (YAN) must levels using four YAN supplementation treatments (T1-T4).

Yeast Strain	EC1118		W15	
YAN	150ppm	250ppm	150ppm	250ppm
T1	1.96^{a}	2.11^{a}	2.38^{b}	2.33^{b}
T2	1.83^{a}	1.86^{a}	2.25^{b}	2.14^{b}
T3	1.52^{a}	1.56^{a}	2.06^{ab}	2.23^{b}
T4	1.52^{a}	1.60^{a}	1.95^{ab}	1.39^{a}

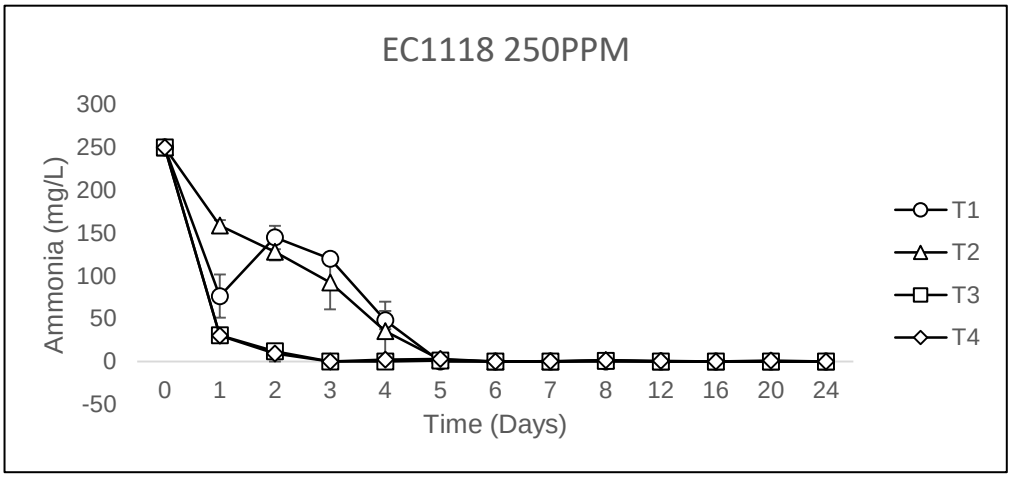
T1 = Diammonium Phosphate (DAP), T2 = DAP+ Fermaid O, T3 = amino acids, T4= Fermaid O
Within a yeast strain, means followed by different letters indicate significant difference from each other using Tukey's test (p<0.05).

Riesling Wine YAN Consumption. In all fermentations, AMM was consumed more rapidly than PAN, but the consumption pattern differed by strain and nutrient type (Figure 1.4, 1.5) T3 and T4 AMM was exhausted more rapidly than T1 and T2 in both EC1118 and W15 (Figure 1.4). W15 consumed AMM faster than EC1118 at both 150ppm and 250ppm. In both strains, AMM was exhausted more rapidly at the lower supplementation level (150ppm) than the higher supplementation level (250ppm) (Figure 1.4).

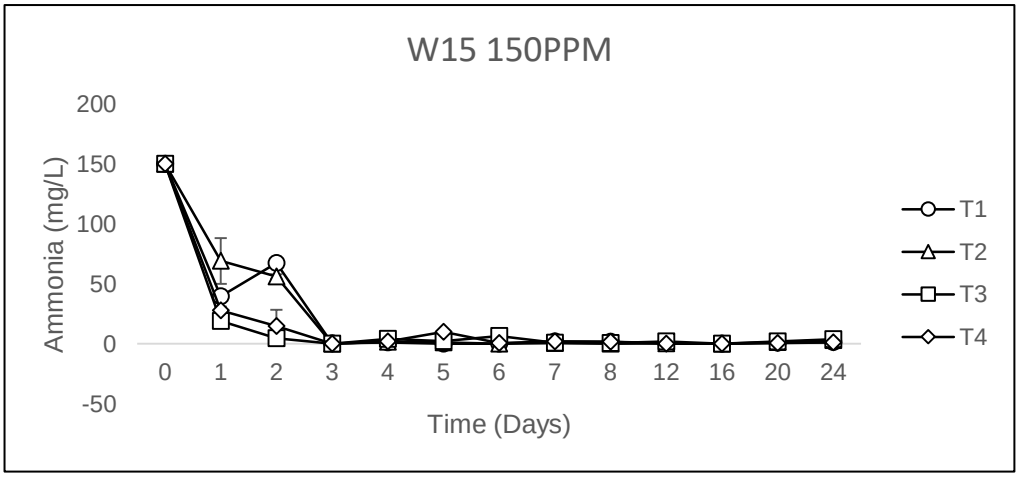
A)


EC1118 150PPM
Ammonia (mg/L)
200
150
100
50
0
-50
0 1 2 3 4 5 6 7 8 12 16 20 24
Time (Days)
T1
T2
T3
T4

B)


EC1118 250PPM
Ammonia (mg/L)
300
250
200
150
100
50
0
-50
0 1 2 3 4 5 6 7 8 12 16 20 24
Time (Days)
T1
T2
T3
T4

C)


W15 150PPM
Ammonia (mg/L)
200
150
100
50
0
-50
0 1 2 3 4 5 6 7 8 12 16 20 24
Time (Days)
T1
T2
T3
T4

D)

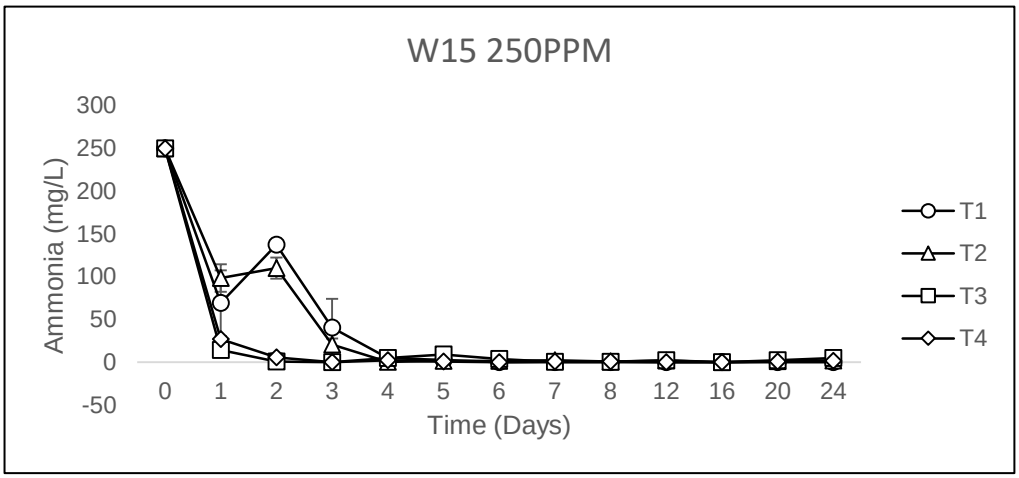
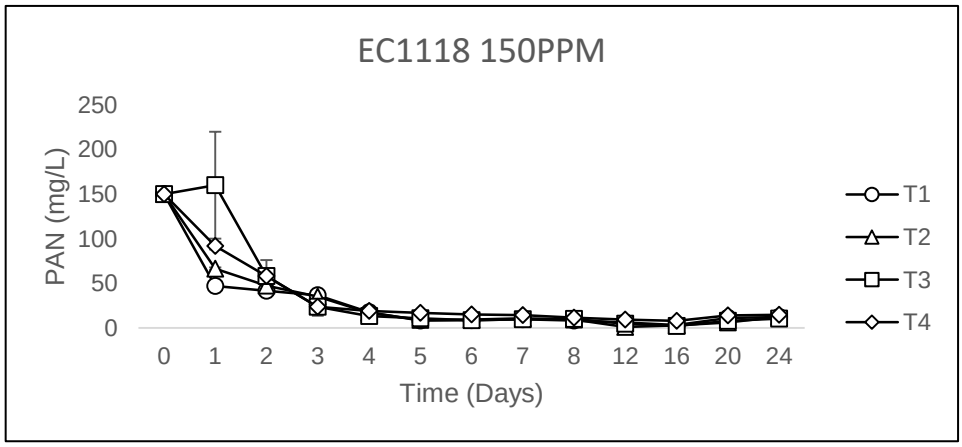


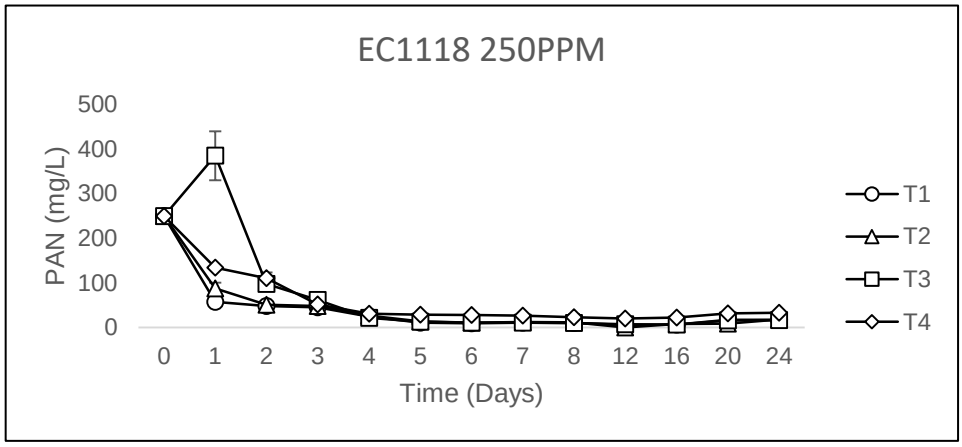
Figure 1.4 Ammonia consumption during fermentation of Riesling wine by EC1118 (A & B) and W15 (C & D). Wines underwent four different nutrient treatments (T1-T4) at two different supplement levels, 150ppm (A & C) and 250ppm (B & D). Error bars represent Standard Error between two fermentation replicates. *T1 = DAP, T2 = DAP +Fermaid O, T3 = amino acids, T4= Fermaid O.*

In EC1118, PAN was initially consumed more slowly in T3 and T4, but was totally exhausted in all treatments by Day 5. W15 had a similar pattern, but consumed PAN more rapidly than EC1118, exhausting PAN by Day 4 (Figure 1.5).

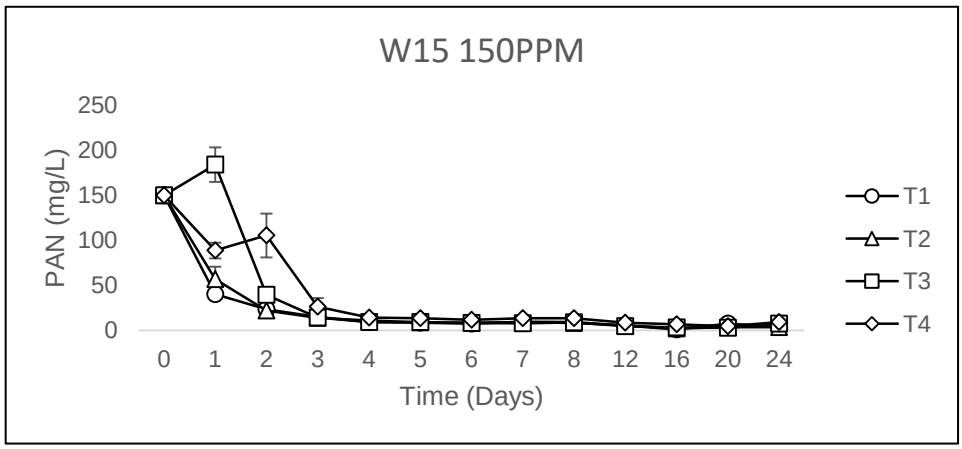
A)



B)



C)



D)

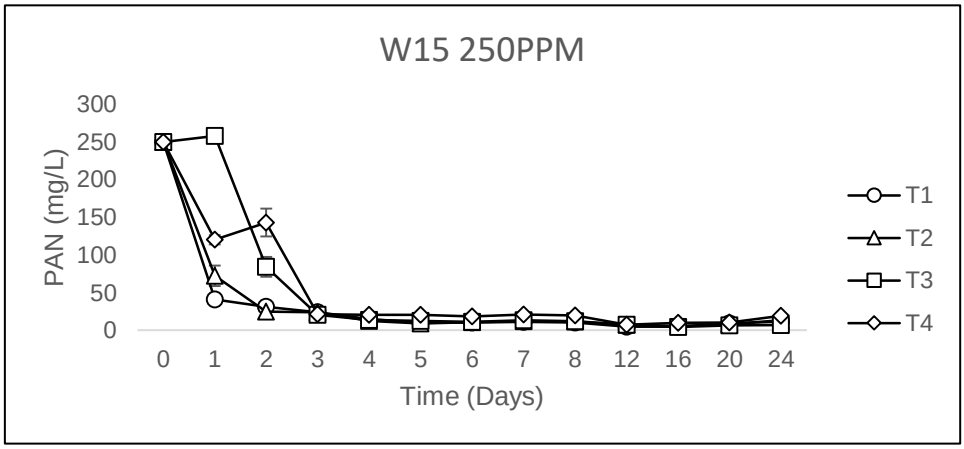


Figure 1.5 Primary Amino Nitrogen consumption during fermentation of Riesling wine by EC1118 (A & B) and W15 (C & D). Wines underwent four different nutrient treatments (T1-T4) at two different supplement levels, 150ppm (A & C) and 250ppm (B & D). Error bars represent Standard Error between two fermentation replicates.*T1 = DAP, T2 = DAP +Fermaid O, T3 = amino acids, T4= Fermaid O.*

Napping® Sensory Study. In EC1118, MFA showed Dimension 1 accounted for 26.84%, and Dimension 2 for 14.73%, of the overall variance (Figure 1.6). There was a strong correlation coefficient between Dimension 1 and T4/250ppm, which also corresponded with the PCA loading of the supplementary variable of 'faulted'. At the 150ppm supplementation rate, T2-T4 corresponded with the attributes of 'mineral' and 'balanced'. T1 treatments at both 150ppm and 250ppm corresponded with the attributes 'astringent' and 'medium acid', but there was a fermentation replicate of T1250ppm that was not grouped with the other samples according to the consensus map. (Figure 1.6). Treatment 2 at 250ppm corresponded with the most responses for 'fruity' and 'high acid'.

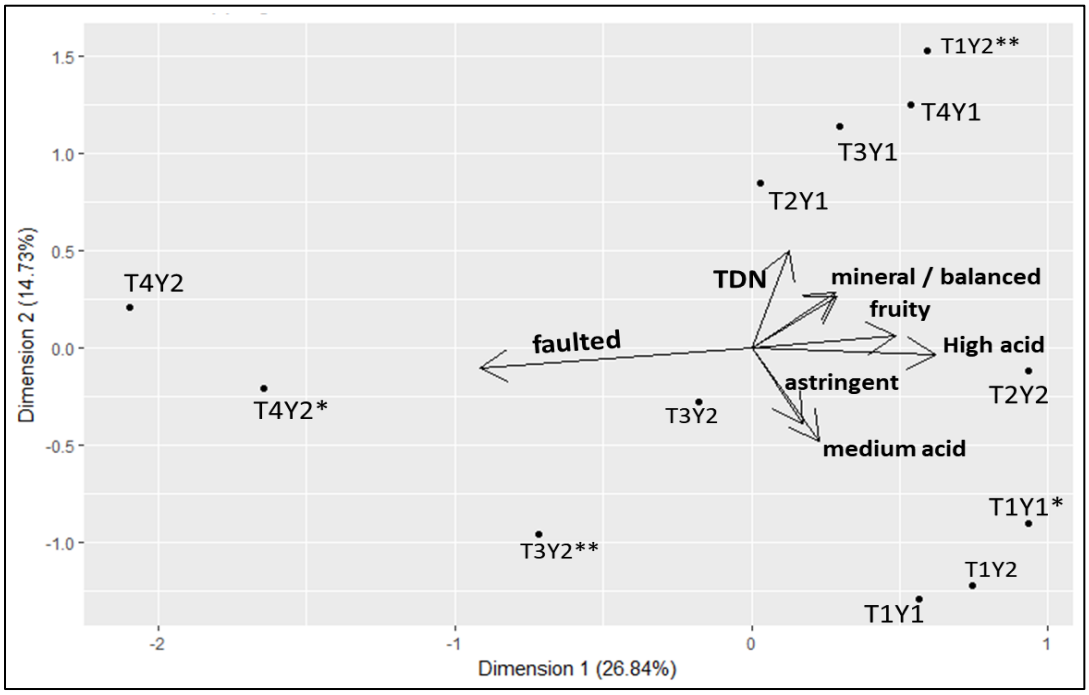


Figure 1.6 Four treatments of Riesling wine fermented with EC1118 at two nitrogen supplementation levels (150 and 250 ppm N) and analyzed by an expert panel using Napping®.
T1= DAP,T2= DAP + Fermaid O, T3 = Amino Acids, T4 = Fermaid O.
** = Sample replicate*
*** = Fermentation replicate*

For W15, Dimension 1 accounted for 26.18% and Dimension 2 for 17.05% of overall variance (Figure 1.7). Similar to EC1118, there was a strong correlation between Dimension 1 and T4 at 250ppm, which also corresponded with the attribute 'faulted'. The treatements, T2-T4 were grouped together at both 150ppm and T1-T3 were all grouped together at 250ppm, but the only associated attribute in W15 was

'medium acid' for the treatments at 150ppm. T1 at 150ppm was also grouped more closely with the other treatments supplemented with organic nutrients at 250ppm. There was an association with T1 in Dimension 1 and the attributes 'astringent' and 'fruity', but this result was not consistent across fermentation replicates (Figure 1.7).

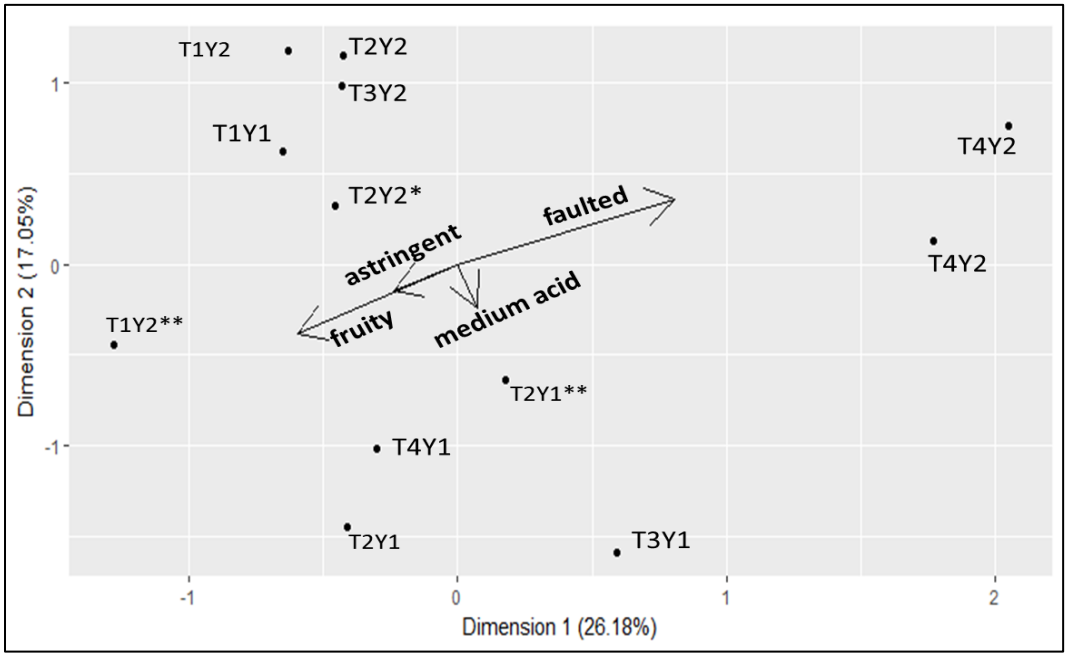


Figure 1.7 Four treatments of Riesling wine fermented with W15 at two nitrogen supplementation levels (150 and 250 ppm N) and analyzed by an expert panel using Napping®.
Riesling wines received four nutrient treatments: DAP (T1), DAP + Fermaid O (T2), Amino Acids (T3), Fermaid O (T4) at two supplementation levels 150ppm N and 250ppm N.
** = Sample replicate*
*** = Fermentation replicate*

Discussion

Model Juice Chemistry.

This study found that nutrient type affected the chemistry of fermented model wine, and that the impact of adding a given nutrient type often corresponded to the supplementation level. In both strains, added nutrients resulted in lower TA, and the reduction in TA was often greater at 250ppm. Lowered TA in response to nutrient supplementation could be attributed to lower potassium uptake by yeast strain, which could result in less potassium bitartrate precipitation, but in model wine tartaric levels and TA were

not well correlated (Tables 1.1, 1.2). The potassium added to the model wine pre-fermentation was most likely the cause of the low levels of tartaric acid after the fermentation, but potassium uptake by yeast was not measured during the course of fermentation.

Model Wine Fermentation Kinetics. YAN was exhausted by Day 4 of the fermentation by both strains at both supplementation levels, with little difference in YAN consumption pattern between treatments. Typically ammonium is the preferred source for biomass accumulation early in fermentation, while amino acids are preferentially utilized in the stationary phase (Bell and Henschke 2005), but the fact that total YAN consumption did not vary widely at a given supplementation level might indicate that these two supplementation levels were not high enough to induce a change in metabolism via NCR.

Though sugar consumption varied with strain and nutrient type, nutrient types containing organic nitrogen (T3, T4) enabled faster kinetics compared to the DAP-based nutrients (T1, T2), which were routinely slower in terms of sugar consumption and higher in residual sugar (Figure 1.1, Table 1.12). The increase in fermentation kinetics observed with organic nitrogen could be attributed to the increase in PAN relative to ammonium (Figure 2). PAN can be more readily incorporated into amino acid via anabolism, whereas ammonium transformation into the required amino acids can be more metabolically costly (Bell and Henschke 2005, Henschke and Jiranek 1993).

Riesling wine chemistry. Nutrient treatments containing only organic nitrogen sources (T3, T4) resulted in a higher pH and lower TA, with greater effect at the higher supplementation rate. The changes in pH and TA can be accounted for by the final levels of tartaric and malic acids (Table 1.6, 1.7, 1.10, 1.11). Organic nutrient sources generally had lower levels of both acids than the inorganic DAP-based nutrients (T1, T2), with the notable exception of W15 T1 at 250ppm. Proton excretion by yeast cells increases in the presence of ammonium ions, and this could explain the lower pH and higher TA evident in T1 and T2 (Pena, Pablo Pardo and Ramirez 1986). Finally, the irregular response of W15 for TA could be due to the fact that this strain is known to be a high succinic acid producer (Scott Labs Handbook 2016), and succinic acid accumulation can raise TA (Bell and Henschke 2005).

Acetic acid production responded to nutrient type in all wines, with lower acetic acid levels corresponding to higher supplementation levels, but with significantly less acetic acid production for wines supplemented with inorganic nitrogen-based sources (Table 1.9). This suggests that inorganic nitrogen sources may be favorable for winemaking when lower pH, higher TA, and lower acetic acid production are the preferred winemaking outcomes. The chemical parameters of T2, which contained some organic nutrients but contained a majority DAP, fell between the wholly organic nutrients (T3, T4) and DAP, and may be more appropriate for winemakers who want to moderate the changes to wine chemistry from nutrient sources.

Riesling Wine Fermentation Kinetics. Nutrient treatments that contained only organic nitrogen sources resulted in as faster fermentations at a given supplementation level (Figure 1.4). Furthermore, fermentation kinetics were higher at the higher supplementation level. Nutrients containing any organic nutrients (T2-T4) did achieve lower residual sugar (RS) than DAP. All supplementation rates and nutrient types achieved dryness for EC1118, but only Fermaid O at 250ppm enabled W15 to achieve dryness, as it is known to behave similarly to AWRI 796 (Maury) (Deed et al. 2011), having a high nitrogen demand and requiring 300mg N/L or greater to achieve dryness (Vilanova et al. 2007). The kinetics and final residual sugar levels agree closely with a previous study that indicated that EC1118 was able to achieve dryness with only 150ppm N supplementation, but W15 did not achieve dryness in response to nitrogen supplementation (Tahim et al. unpublished). Overall, Fermaid O not only resulted in more rapid fermentation kinetics for both strains but resulted in lower residual sugar, suggesting that use of this organic nitrogen source could increase fermentation efficiency in a winery environment.

Riesling wine sensory outcomes. The general consensus among panelists that wines fermented using Fermaid O at 250ppm were ‘faulted’ suggests that this level, which is well above the legal limit for Fermaid O additions, would not be viable as a fermentation nutrient for quality wine production. Wines supplemented to 150ppm with some level of organic nutrients (T2-T4) had desirable sensory attributes of

'balanced', 'mineral', 'petrol', 'medium acid', but the 250ppm level was not associated with the same desirable attributes. In both strains, DAP-supplemented wine (T1) was associated with 'astringent', which could be related to the higher TA observed with the use of that nutrient type (Table 1.7). It is important to note that only wines supplemented to 250ppm with T2 in EC1118 and T1 in W15 were associated with 'fruity', which might indicate an increase in ester formation, but these wines also had less desirable mouthfeel characteristics.

In terms of optimization of both fermentation and sensory parameters, T2 had final chemistry and kinetics that more closely resembled DAP alone, but it was associated with 'fruity' in EC1118, rather than 'astringent' as seen with DAP alone. The chemistry attributes associated with DAP-based nutrients (T1 and T2) and the increased fermentation kinetics associated with the presence of Fermaid O suggests that T2 (Fermaid O+DAP) may help realize both beneficial wine chemistry and desirable sensory outcomes. It is worth noting that increasing supplementation to 250ppm may not increase desirable sensory outcomes, and based on strain and nutrient choice, may have fewer desirable sensory characteristics (Figure 1.5, 1.6).

Conclusion

The use of organic nitrogen during the course of YAN supplementation can have important implications for both wine chemistry and sensory outcomes in the fermentation of cool-climate Riesling. Important wine chemistry parameters, including pH, TA, and acetic acid, can be directly affected by the choice of nutrient package. While organic nutrient sources can increase fermentation kinetics, they can also result in higher pH and acetic acid and lower TA, so winemakers should balance their choice of nutrient type based on the desired chemistry of the final wine. Organic nutrients can improve the ability of a strain to reach dryness, but 250ppm N may not be sufficient YAN in strains with high nitrogen demand. A combination of Fermaid O and DAP can help realize an increase in kinetics, while still preserving the lower pH and acetic acid of a DAP-only supplementation regime. In terms of sensory outcomes, a lower supplementation level of 150 ppm appears to be associated with more desirable attributes. Nutrient

regimes containing only DAP also appear to have less desirable attributes, indicating that a combination of Fermaid O and DAP supplemented to 150ppm N can achieve beneficial chemistry parameters and desirable sensory outcomes in the course of cool-climate Riesling fermentation, especially when using a yeast strain with lower nitrogen needs.

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CHAPTER 3:

YEAST ASSIMILABLE NITROGEN SOURCE AND FERMENTATION TEMPERATURE AFFECT THE CHEMISTRY AND SENSORY PROPERTIES OF COOL CLIMATE RIESLING

Abstract

Supplementation of Riesling fermentations with organic vs. inorganic nitrogen may affect wine chemistry, nitrogen uptake, and perceived wine quality. During alcoholic fermentation of grape juice, nitrogen supply and fermentation temperature influence volatile composition and resultant wine quality. A sufficient supply of Yeast Assimilable Nitrogen (YAN) is essential not only to avoid stuck or sluggish fermentations, but also to facilitate yeast metabolic processes that can produce desirable aroma compounds. As a result, YAN is regularly monitored and supplemented during commercial winemaking, typically by adding inorganic nitrogen in the form of Diammonium Phosphate (DAP). In a study comparing two yeast strains (EC1118 and W15), fermentation temperature was found to have a greater had an impact on both fermentation kinetics and dryness than nutrient source. Yeast strain, nutrient source, and fermentation temperature all affected key chemistry metrics such as pH, titratable acidity, acetic acid, and malic acid levels in the final wines. A panel of wine experts analyzed the sensory properties of the wines, using a projective mapping technique known as Napping®. Desirable sensory attributes such as ‘fruity’, ‘balanced’, ‘candied’ and ‘clean’ were most closely associated with lower fermentation temperatures. As a result, perceived wine quality may be enhanced by utilizing lower fermentation temperature, but increased YAN supplementation may be required in order to achieve dryness in some yeast strains.

Introduction

Even in a glucose-rich medium like grape juice, alcoholic fermentation can be limited by a lack of nitrogen. Yeast Assimilable Nitrogen (YAN), the nitrogenous fraction available to microbial metabolism, is necessary for both yeast biomass accumulation and metabolism (Bell and Henschke 2005). If

insufficient YAN is present, fermentation can become stuck or sluggish (Bisson and Butzke 2000). Additionally, low YAN can alter the metabolic pathways that biosynthesize amino acids, which can result in hydrogen sulfide (H_2S) formation (Bell and Henschke 2005). H_2S has an unpleasant rotten egg aroma, and is considered detrimental to wine quality. In order to ensure successful and rapid fermentation completion and prevent the formation of off-aromas, YAN is routinely supplemented during commercial winemaking.

YAN supplementation impacts the production of volatile compounds including fatty acids, fusel alcohols, and esters (Bell 2005). Inorganic nitrogen (AMM), in the form of Diammonium Phosphate (DAP), is the most common additive. Inorganic ammonium is the preferred by yeast for biomass formation, but primary amino nitrogen (PAN) from amino acids is preferentially used later, during the stationary phase, for cell maintenance (Beltran et al. 2005). The presence of DAP can induce Nitrogen Catabolite Repression (NCR), a process by which yeast regulate their uptake of amino acids by changing the expression of permeases responsible for nitrogen transport into the cell. The changes in the expression of permeases is modulated according to the availability of nitrogen sources that are easiest to metabolize, such as ammonia (Beltran et al. 2004). Wines supplemented with organic nitrogen, on the other hand, or a combination of ammonium and organic nitrogen, have higher concentrations of acetate and ethyl esters (Torrea et al. 2011, Barbosa et al. 2012).

Fermentation temperature can also influence the volatile composition of the final wine. Yeast membrane fluidity decreases with lower temperatures, as yeast modify the degree of saturation in their lipid bilayer. This change in degree of lipid saturation is achieved by increasing synthesis of polyunsaturated fatty acids (PUFA) versus saturated fatty acids (Torija et al. 2003). PUFA synthesis is an oxidative process, and in the reductive environment of winemaking, this operation is interrupted and mid-chain fatty acids (MCFA) are released into the medium. MCFA can be transformed via both enzymatic esterification as part of yeast metabolism or by acid-catalyzed esterification reactions in the presence of ethanol, forming ethyl esters, which are potent fruity aromas with low sensory thresholds (Waterhouse et al. 2016, Saerens et al.

2010). During the stationary phase, the activity of the genes regulating enzymes responsible for ester formation are affected by temperature. ATF1 and ATF2, responsible for acetate ester formation, are more active at lower temperatures, whereas those responsible for ethyl ester formation, EEB1 and EHT1, are more active at higher temperatures, particularly above 18°C (Mouret et al. 2014). There are physical effects of fermentation temperature on the medium that can alter ester composition as well. Lower fermentation temperature can lower the amount of volatile esters lost as a result of the changes to the gas-liquid phase ratio as well as compound volatility and subsequent CO_2 entrainment (Mouret et al. 2012).

Fermentation temperature can also have an impact on nitrogen uptake and consequent changes in the volatile aroma composition of the final wine. The membrane permeases responsible for YAN transport can undergo structural changes based on temperature (Entian and Barnett 1992). Both yeast biomass and nitrogen consumption increase at higher temperatures, and are more pronounced when ammonium is the sole nitrogen source. Permease repression by NCR is less effective at lower temperatures due to the decreased fluidity of the plasma membrane and permease functionality, meaning that low temperature fermentations are metabolically similar to low YAN fermentations (Beltran et al. 2007). Though temperature does alter permease activity, temperature does not dramatically alter the metabolic pathways for ester production. In fact, the largest impact of temperature on volatile composition is not a significant metabolic change, but evaporation alone (Mouret et al. 2014, Rollero et al. 2015). While temperature does not independently alter yeast nitrogen metabolism and volatile ester synthesis, temperature and nitrogen uptake can have an interactive effect. The length of the growth phase of yeast does impact the total amount of volatile compounds produced, and the length of that growth phase is altered by both initial nitrogen content and temperature. Higher temperatures result in faster fermentation, but higher initial nitrogen content can extend the length of the growth phase (Mouret et al. 2014). Nitrogen metabolism is known to regulate the quantity of several yeast metabolites, so nitrogen supplementation often alters final wine chemistry. Previous studies have compared compositional changes from inorganic versus organic nitrogen supplementation primarily by measuring fermentation kinetics and volatile

production, but additional work is needed in identifying the interactive effects of nutrient and temperature and their implications on wine sensory qualities. This study sought to elucidate the effects of nitrogen of inorganic versus organic nitrogen source and fermentation temperature on both wine composition and sensory qualities. Given the differences produced by varying quantitative and qualitative YAN supplementation, and the interactive effect of temperature, this study focused on optimizing the fermentation parameters for typically YAN-deficient Riesling from the Finger Lakes region of New York.

Materials and Methods

Fruit Processing. Riesling grapes were harvested at Cornell University's Lansing Research Vineyards in the Finger Lakes of New York on October 3rd, 2016, once vineyard sampling showed the fruit had reached 21º Brix. A total of 1,043.3kg of fruit were hand harvested to exclude *Botrytis cinerea* infected clusters, collected into picking bins, and transported to the Cornell Orchards winery in Ithaca, NY. Bins were emptied into a destemmer (Bucher Delta E1, Rivesaltes, Switzerland) and pumped into a membrane press (Scharfenberger Tx3 Europress, Bad Dürkheim, Germany). The fruit was divided into 4 press loads, which were pressed in an increasing pressure stage program up to a maximum of 1.7 bars for a total of 140 minutes per press load. 40mg/L of sulfur dioxide (SO_2) was added into the crush pan during each press iteration in the form of potassium metabisulfite ($K_2S_2O_5$) in order to prevent oxidation of the juice. Juice was collected into a 750L plastic tank, mixed to homogenize, and was allowed to settle overnight at 2.8º C. The following day, 11.3L aliquots of juice were racked into thirty-six 19L glass carboys.

Yeast Selection. Each fermentation treatment was inoculated with one of two yeast strains. *Saccharomyces bayanus*, EC1118 (Lallemand, Montreal, CA), and *Saccharomyces cerevisiae*, W15 (Lallemand, Montreal, CA), were chosen due to their popularity in the Finger Lakes for Riesling fermentation as well as their differing nitrogen requirements, as noted by the manufacturer.

Nitrogen Supplementation and Fermentation Temperature. Fermentations were initiated with a YAN concentration (calculated as the sum of PAN and AMM) adjusted to 150ppm, which is traditionally considered to be near the minimum required for successful fermentation (Bell and Henschke 2005). YAN was supplemented using either inorganic nitrogen, in the form of DAP, or organic nitrogen, in the form of a food-grade amino acid mixture (Sigma-Aldrich, St. Louis, MO, USA). The amino acid mixture was composed of amino acids mixed in proportion those found in Riesling (Spayd and Andersen-Bagge 1996). The experimental design consisted of two yeast strains receiving separate nutritional regimes of inorganic nitrogen (DAP) or organic nitrogen (amino acids), and a non-supplemented control (77mg/L). These three nutritional treatments were then fermented at three different temperatures: 23° C, 18° C, and 12° C. All treatments were performed in duplicate.

Sampling. For each fermentation, duplicate 2mL samples were taken every other day from each carboy, using 25mL disposable sterile pipettes (Celltreat, Shirley, MA, USA.) Samples were stored in 2mL Eppendorf tubes at -15° C until analyzed.

Analytical methods. The concentrations of sugars (glucose and fructose) and organic acids (tartaric, malic, citric, lactic, and acetic) were quantified using high performance liquid chromatography (HPLC) on an Agilent Systems 1100 HPLC (Agilent Technologies, Santa Clara, CA, USA), equipped with photodiode array detector and refractive index on Cation Exchange Column (Bio-Rad Aminex HPX-87H, 300mm x 7.8mm). The analysis method was isocratic at 0.5mL/min, using 6% acetonitrile in 0.045N sulfuric acid mobile phase and a column temperature of 45° C. YAN was calculated using a Chemwell 2910 Multianalyzer (Unitech Scientific, Hawaiian Gardens, CA, USA) to perform separate enzymatic tests of primary amino nitrogen (PAN) and ammonia (AMM). AMM was quantified with a glutamate dehydrogenase enzymatic assay (Ough 1969), using an enzymatic kit (Unitech Scientific, Ammonia Extended Range UniTAB, 2007). PAN was determined by a N-acetylcysteine/ o-phthaldehdye spectrophotometric assay (NOPA) (Dukes and Butzke 1998), using an enzymatic kit (Unitech Scientific, Primary Amino Nitrogen UniTAB, 2007).

Sensory evaluation. Wines were evaluated using a modified projective mapping technique, known as Napping®, using a panel of 17 wine professionals, consisting of 7 males and 10 females, aged 21-70 years. The panelists executed a Napping® Test (Pagès 2005) on a 60x40cm sheet of white butcher paper, such that similar samples were placed physically close together and different samples were placed physically far apart.

Wines were evaluated using a modified projective mapping technique, known as Napping®, using a panel of 17 wine professionals, including winemakers and extension personnel. The panel consisted of 8 males and 9 females, aged 21-66 years. Panelists convened at the tasting room of Fox Run Vineyards (Penn Yan, NY). Fifty mL wine samples were served in matching 300mL ISO tasting glasses labeled with three-digit random numbers, covered with petri dishes, and presented to panelists in balanced order dictated by Williams Latin Square. Water, spit cups, paper napkins, and unsalted crackers were provided to the panelists. The panelists were then asked to execute a Napping® Test (Pagès 2005) on a 60cmx40cm white butcher paper, where they were instructed to place similar samples physically close together and different samples were placed physically far apart. The criteria for similarity was self-derived for each panelist, according to the methods outlined in Pagès et al. 2005. Each flight contained wines (50mL) fermented with only one yeast strain, and they contained one sample from each of the wines fermented with the three nutrient treatments and three fermentation temperatures in that given strain, comprising eight of the twelve samples. There were four controls in each flight, two samples were from fermentation replicates and two were sample replicates, identical to another sample in the flight. For each panelist, samples order was balanced using a Williams Latin Square to mitigate bias.

Statistical Analysis. Statistical analysis of wine chemical parameters and sensory evaluation was performed using R Studio version 3.3.3 (R Foundation for Statistical Computing, Vienna, Austria). Wine chemistry parameters were analyzed using a linear model, predicting means based on a full factorial design of two nutrient treatments plus control, three fermentation temperatures, and two yeast strains

creating a 3x3x2 ANOVA. Post-hoc analyses and estimated predicted means were calculated with the R package lsmeans (Lenth 2016).

Analysis of the Napping® sensory data was performed using the R packages SensoMineR (Le and Husson 2008) and FactoMineR (Lê, Josse and Husson 2008) computing environments. The Euclidean distances of the wine samples were compiled into a consensus configuration and analyzed using Multi-Factor Analysis (MFA) (Morand and Pagès 2006). The consensus configuration was derived by performing a PCA on the panelists' arrangement of the individual wine samples by X and Y coordinates on the nappe, which were then normalized between panelists by dividing all of the components by the first eigenvalue. A second PCA was then performed on the normalized data to generate the consensus map, and a Procrustes rotation, based on the RV coefficient's statistical measure of fit between configurations, then allowed for maximum agreement between panelists' configurations. The final location on the PCA plot represents the Procrustes rotation of the consensus map. The descriptors were compiled into attribute counts and treated as supplementary variables. The supplementary variables were represented as a PCA model based on their correlation coefficient with each axis of the sample loadings (Perrin et al. 2008). Only statistically significant attribute counts were utilized in the PCA model.

Results

Initial Riesling Juice Chemistry. Following crush, Riesling juice was found to have 21º Brix soluble solids, a pH of 3.42, and titratable acidity (TA) of 6.0g/L (in TAE). AMM was 26mg/L and PAN was 34 mg/L, making a total YAN of 60mg/L.

Effects of Nutrient Type and Temperature on Riesling Wine Chemistry.

Organic and inorganic nutrient supplementation resulted in differences in key wine chemistry parameters, such as pH, TA, and acetic acid production. The two yeast strains exhibited similar responses in pH at when fermented at the same temperatures, with pH generally lower at higher fermentation temperatures (Table

2.1). Within a given strain, no factor resulted in a significant change in pH, but there were differences in pH across the two yeast strains pH for both temperature and nutrient choice ($p<0.05$).

Table 2.1 Mean pH in Riesling wines produced with two yeast strains levels using three YAN supplementation treatments – control (no addition), Diammonium Phosphate (DAP) to 150ppm, or amino acids to 150ppm, and fermented at 23º C, 18º C, or 12º C.

Yeast Strain	EC1118			W15		
	Control	DAP	Amino Acids	Control	DAP	Amino Acids
23º C	3.35^{a}	3.30^{a}	3.29^{a}	3.27^{a}	3.27^{a}	3.31^{a}
18º C	3.34^{a}	3.32^{a}	3.37^{a}	3.31^{a}	3.29^{a}	3.33^{a}
12º C	3.31^{a}	3.32^{a}	3.37^{a}	3.32^{a}	3.29^{a}	3.35^{a}

Within a yeast strain, means followed by different letters indicate significant difference from each other using Tukey's test ($p<0.05$).

Table 2.2 Mean titratable acidity[1] (g/L) in Riesling wines produced with two yeast strains levels using three YAN supplementation treatments – control (no addition), Diammonium Phosphate (DAP) to 150ppm, or amino acids to 150ppm, and fermented at 23º C, 18º C, or 12º C.

Yeast Strain	EC1118			W15		
	Control	DAP	Amino Acids	Control	DAP	Amino Acids
23º C	6.79^{a}	6.55^{a}	6.29^{a}	8.18^{a}	7.58^{a}	7.23^{a}
18º C	6.67^{a}	6.67^{a}	6.32^{a}	7.88^{a}	7.57^{a}	7.26^{a}
12º C	6.60^{a}	6.73^{a}	6.62^{a}	6.66^{a}	6.69^{a}	7.06^{a}

[1]Expressed as Tartaric Acid Equivalents (TAE)
Within a yeast strain, means followed by different letters indicate significant difference from each other using Tukey's test ($p<0.05$).

Table 2.3 Mean acetic acid (g/L) in Riesling wines produced with two yeast strains levels using three YAN supplementation treatments – control (no addition), Diammonium Phosphate (DAP) to 150ppm, or amino acids to 150ppm, and fermented at 23º C, 18º C, or 12º C.

Yeast Strain	EC1118			W15		
	Control	DAP	Amino Acids	Control	DAP	Amino Acids
23º C	0.44^{cd}	0.26^{ab}	0.52^{d}	0.35^{ab}	0.34^{ab}	0.34^{ab}
18º C	0.43^{bcd}	0.11^{a}	0.38^{bcd}	0.36^{ab}	0.29^{a}	0.38^{ab}
12º C	0.54^{d}	0.34^{bc}	0.49^{cd}	0.42^{ab}	0.44^{ab}	0.49^{b}

Within a yeast strain, means followed by different letters indicate significant difference from each other using Tukey's test ($p<0.05$).

Table 2.4 Mean tartaric acid (g/L) in Riesling wines produced with two yeast strains levels using three YAN supplementation treatments – control (no addition), Diammonium Phosphate (DAP) to 150ppm, or amino acids to 150ppm, and fermented at 23º C, 18º C, or 12º C.

Yeast Strain	EC1118			W15		
	Control	DAP	Amino Acids	Control	DAP	Amino Acids
23º C	3.86^{ab}	3.83^{ab}	3.72^{ab}	4.17^{b}	4.18^{b}	4.12^{ab}
18º C	3.62^{a}	3.86^{b}	4.07^{ab}	3.96^{ab}	3.89^{ab}	3.73^{a}
12º C	3.90^{ab}	3.87^{ab}	3.82^{ab}	4.12^{b}	3.99^{ab}	3.84^{ab}

Within a yeast strain, means followed by different letters indicate significant difference from each other using Tukey's test (p<0.05).

Table 2.5 Mean malic acid (g/L) in Riesling wines produced with two yeast strains levels using three YAN supplementation treatments – control (no addition), Diammonium Phosphate (DAP) to 150ppm, or amino acids to 150ppm, and fermented at 23º C, 18º C, or 12º C.

Yeast Strain	EC1118			W15		
	Control	DAP	Amino Acids	Control	DAP	Amino Acids
23º C	2.11^{b}	2.03^{ab}	2.03^{ab}	3.09^{e}	2.31^{bc}	2.47^{d}
18º C	2.12^{b}	1.96^{a}	2.00^{ab}	3.09^{e}	2.44^{cd}	2.48^{d}
12º C	2.03^{ab}	2.03^{ab}	2.05^{ab}	2.27^{b}	2.10^{a}	2.11^{a}

Within a yeast strain, means followed by different letters indicate significant difference from each other using Tukey's test (p<0.05).

Table 2.6 Mean residual sugar (g/L) in Riesling wines produced with two yeast strains levels using three YAN supplementation treatments – control (no addition), Diammonium Phosphate (DAP) to 150ppm, or amino acids to 150ppm, and fermented at 23º C, 18º C, or 12º C.

Yeast Strain	EC1118			W15		
	Control	DAP	Amino Acids	Control	DAP	Amino Acids
23º C	1.01^{ab}	0.76^{a}	0.84^{a}	9.49^{e}	4.99^{a}	0.88^{a}
18º C	1.02^{ab}	0.92^{ab}	0.95^{a}	3.43^{b}	2.19^{a}	0.81^{a}
12º C	9.47^{d}	5.72^{bc}	2.02^{c}	31.19^{f}	19.34^{c}	7.89^{c}

Within a yeast strain, means followed by different letters indicate significant difference from each other using Tukey's test (p<0.05).

Yeast strain contributed to differences in TA ($p<0.001$), and temperature and yeast strain together showed a two-way interaction on TA across the strains ($p<0.02$), but no factor significantly altered TA within a strain (Table 2.2).

Acetic acid production was altered by temperature ($p<0.001$) (Table 2.3), and the impact of temperature could most clearly be seen at 18° C, where there was lower acetic acid production relative to the other two fermentation temperatures for a given strain and nutrient type (Table 2.3). Production of acetic acid was lowest in fermentations supplemented with DAP, with acetic acid levels below the control and

organic nutrients in all cases, except W15 at 12° C. Nutrient package and yeast strain had an interactive affect on acetic acid production ($p<0.001$).

Tartaric acid was affected by the addition DAP, which resulted in generally higher values than the amino acid treatments. Temperature also affected tartaric acid levels, but the changes due to temperature were not consistent across strains (Table 2.4). Malic acid levels were affected by all nutrient conditions and temperatures in W15, but EC1118 malic acid was only affected at 18º C with the addition of DAP, having lower malic acid than other conditions (Table 2.5). Both tartaric and malic acid were affected by the choice of yeast strain ($p<0.01$) with higher levels of both acids evident in W15 fermentations (Tables 2.4, 2.5).

Residual sugar levels were dependent on both fermentation temperature and nutrient supplementation. Lower fermentation temperature resulted in more residual sugar, with up to 31g/L remaining in W15 at 12º C. Nutrient supplementation yielded lower residual sugar than the unsupplemented control, and organic nutrient supplementation routinely had lower residual sugar than inorganic nutrient supplementation (Table 2.6).

Effects of Nutrient Type and Temperature on Fermentation Kinetics.

In both strains, YAN consumption corresponded to fermentation temperature. AMM was exhausted by Day 4 at 23º C and Day 6 at 12º C (Figure 2.1). PAN was consumed at the same rate by both strains at all fermentation temperatures, and was exhausted by Day 2 (Figure 2.2). In both strains, the rate of nutrient exhaustion was identical at 23º C and 18º C, though nutrient uptake was different in fermentations at 23º C and 12º C.

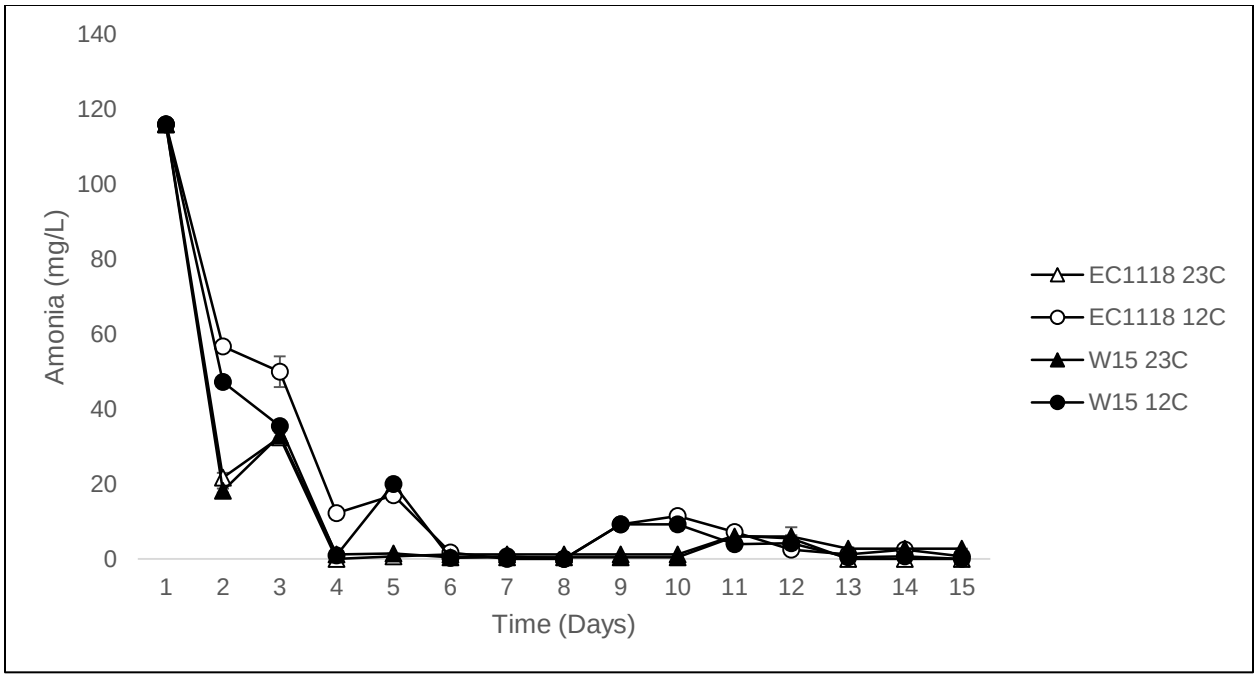


Figure 2.1. Ammonium consumption of Riesling wine supplemented with diammonium phosphate (DAP), fermented with strain EC1118 at 23º C (△) and 12º C (○) and strain W15 at 23º C (▲) and 12º C (●). Error bars (not visible) represent Standard Error between the two sample replicates.

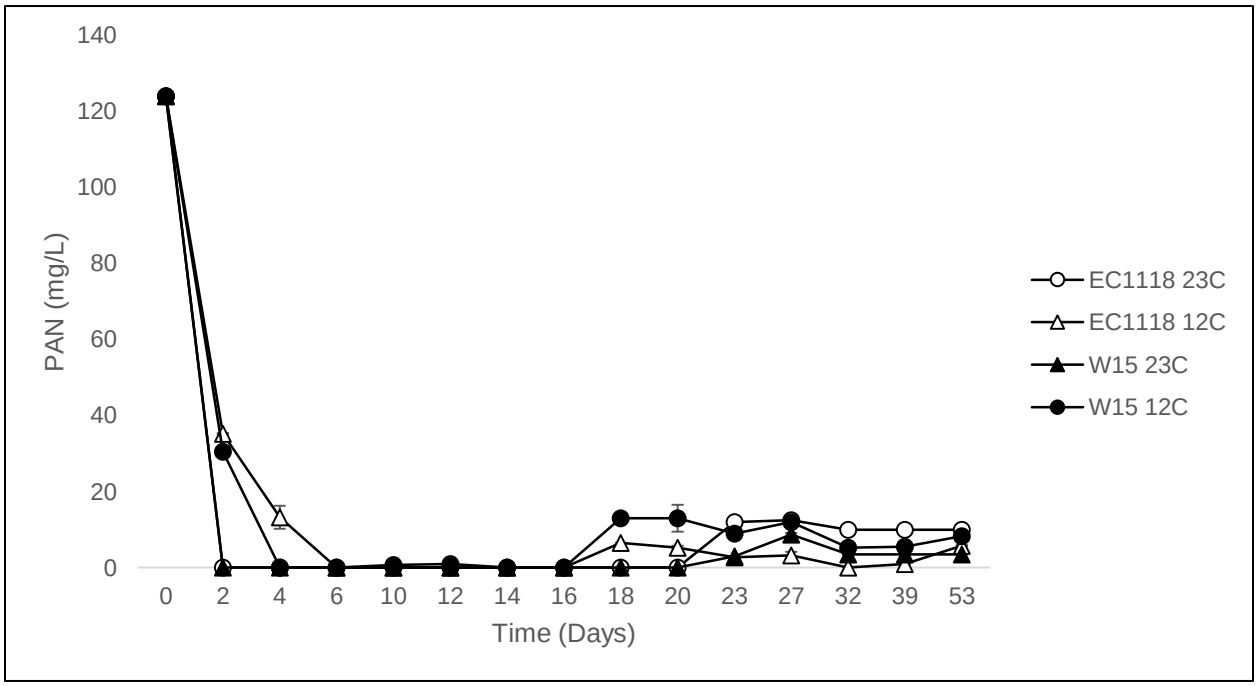
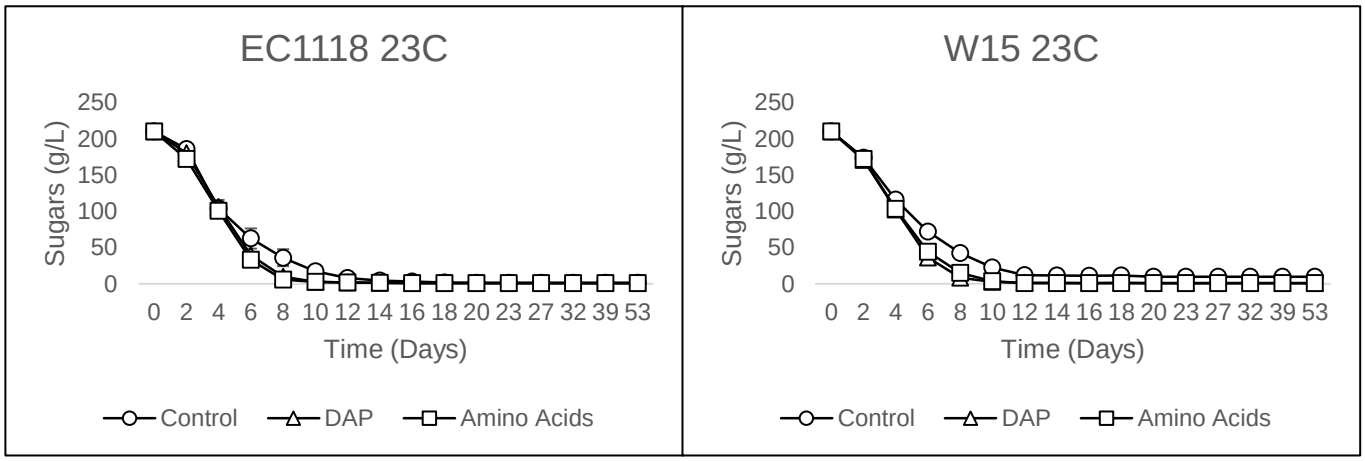


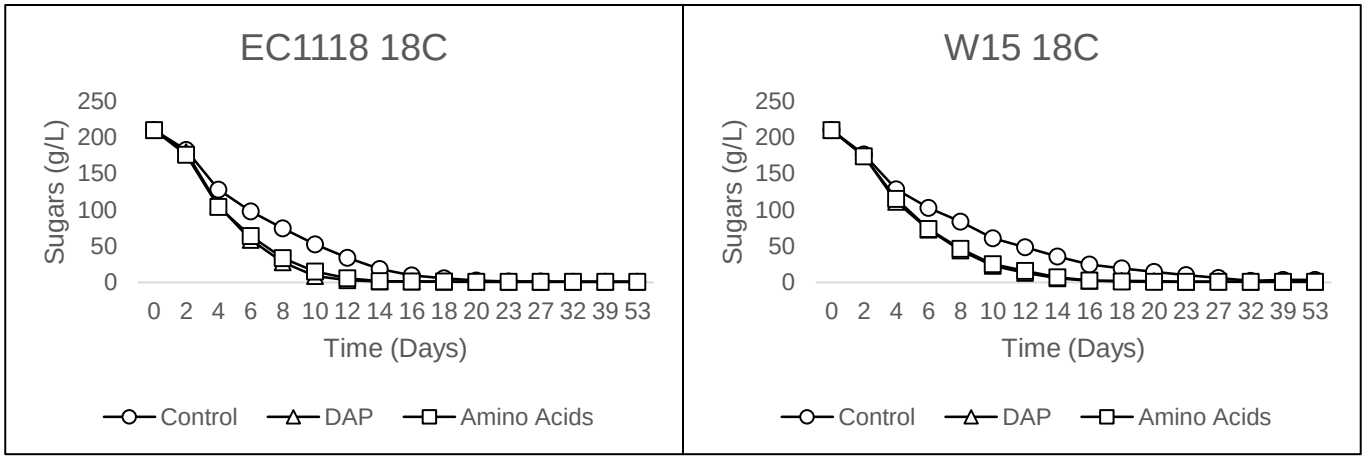
Figure 2.2. Primary amino nitrogen consumption of Riesling wine supplemented with amino acids, fermented with strain EC1118 at 23º C (△) and 12º C (○) and strain W15 at 23º C (▲) and 12º C (●). Error bars represent Stndard Error between the two sample replicates.

A three-way interaction of strain, nutrient, and temperature was observed for sugar consumption and days to completion ($p<0.01$), with fermentation temperature inducing the largest difference in fermentation kinetics. Fermentations at 12º C consumed sugars more slowly than the two warmer fermentations, and the fermentation temperature affected both strains' ability to achieve dryness, particularly W15. In all cases, fermentations at 12º C took more than 40 days to complete fermentation, and did not reach dryness in several cases, particularly that of the unsupplemented control. EC1118 completed fermentation (<2g/L sugar) in all of the low temperature fermentations when provided with either inorganic or organic nitrogen supplementation, but not with the low temperature control. W15 control fermentations, on the other hand, did not achieve dryness at any temperature. In both strains, organic nutrients resulted in equivalent or slightly shorter fermentation times than inorganic nutrients, but both nutrient types resulted in shorter fermentation times than the control (Figure 2.3).

A) **i** **ii.**



B) **i.** **ii.**



C) **i.** **ii.**

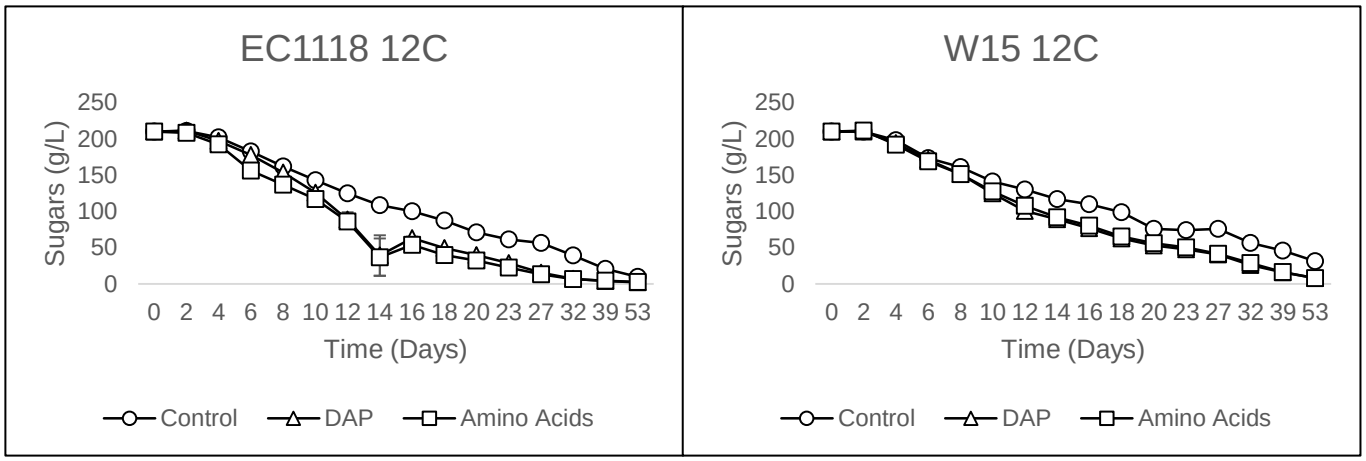


Figure 2.3. Sugar consumption of Riesling wine fermented with EC1118 (i) and W15 (ii) at 23° C (A), 18° C (B), and 12° C (C), with three different nutrient supplementation regimes. Error bars represent the Standard Error between fermentation replicates.

Napping® Sensory Study. For EC1118 fermentations, Dimensions 1 and 2 accounted for 22.57% and 18.22% of the overall variance, respectively, as calculated by MFA (Figure 2.4). Fermentations at 12º C appeared to be the most distinctive grouping, and were clearly separated from the other two fermentation temperatures. Fermentations at 12º C were most closely associated with attributes 'fruity', 'low acid', 'aromatic', and 'balanced'. The unsupplemented control wines fermented at 18º C and 23º C were associated with the attributes 'muted' and 'mineral', whereas both the supplemented fermentations at

23º C corresponded to attributes of 'reduced', 'watery', and 'faulted.' Finally, the 18º C fermentations supplied with inorganic DAP were associated with 'astringent' and the amino acid supplemented fermentations were associated with the attribute 'oxidized' (Figure 2.4).

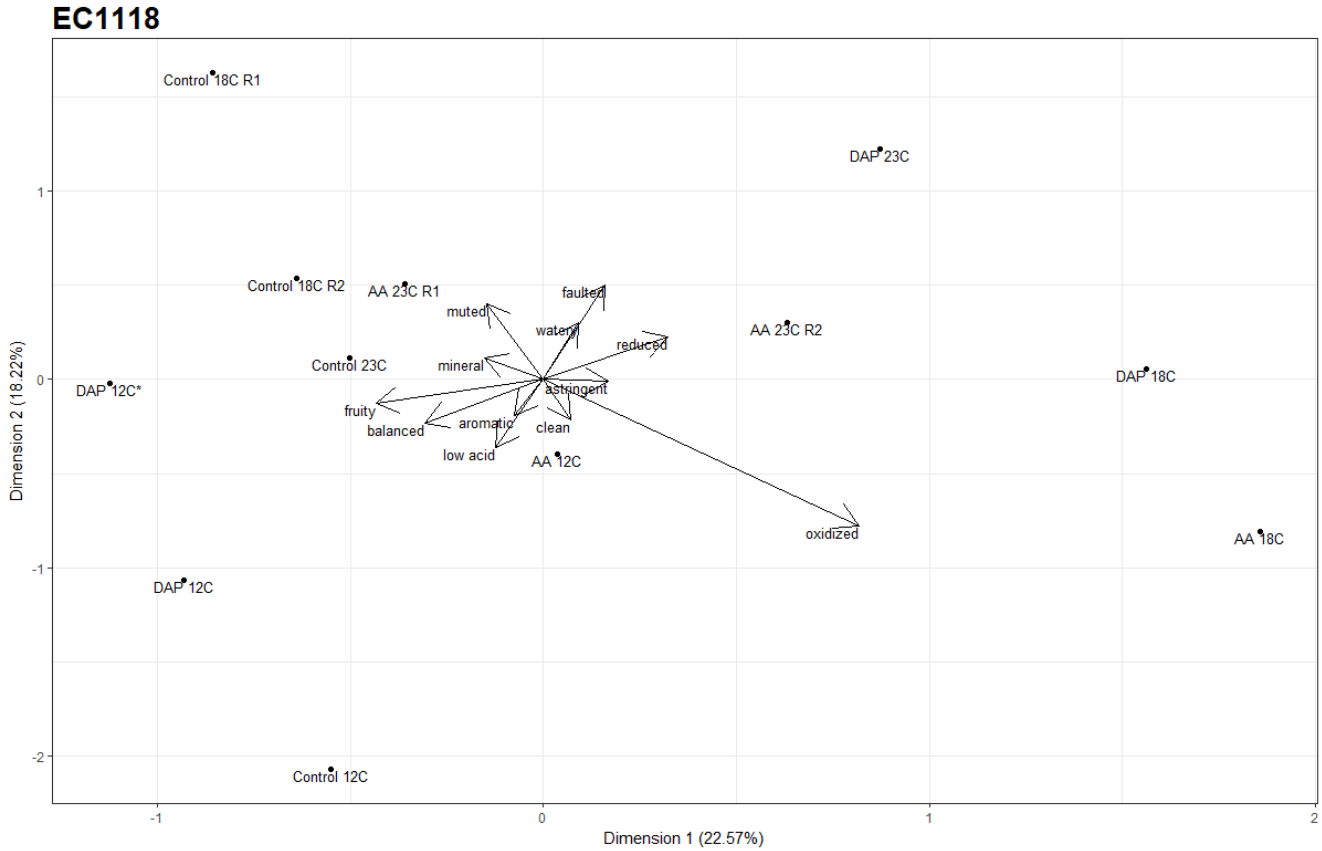


Figure 2.4. Sensory attributes of Riesling wines fermented with EC1118 at three different fermentation temperatures. Fermentations were received different nutrient treatments DAP to 150ppm YAN, amino acids (AA) to 150ppm YAN, or no supplementation (Control). R1 and R2 indicate different fermentation replicates. * indicates sample replicates.

For W15 fermentations, Dimensions 1 and 2 accounted for 28.47% and 22.82% of the overall variance, respectively, as calculated by MFA (Figure 2.5). As with EC1118, fermentations at 12º C were grouped, regardless of nutrient type; and the two supplemented fermentations were described as being 'fruity'. The attribute 'TDN' (1,1,6-trimethyl-1,2-dihydronaphthalene or 'petrol') was associated with the 12º C control, as were 'candied' and 'sweet'. Fermentations at 18º C and 23º C were very closely grouped with the exception of the amino acid supplemented fermentations at 18º C, which was clearly separated from the other samples. The cluster of samples from the 18º C and 23º C fermentations had a range of

associated attributes, including ‘medium acid’, ‘watery’, and ‘muted’. The most closely associated attribute to the grouped amino acid fermentations at 18º C was ‘floral’ (Figure 2.5).

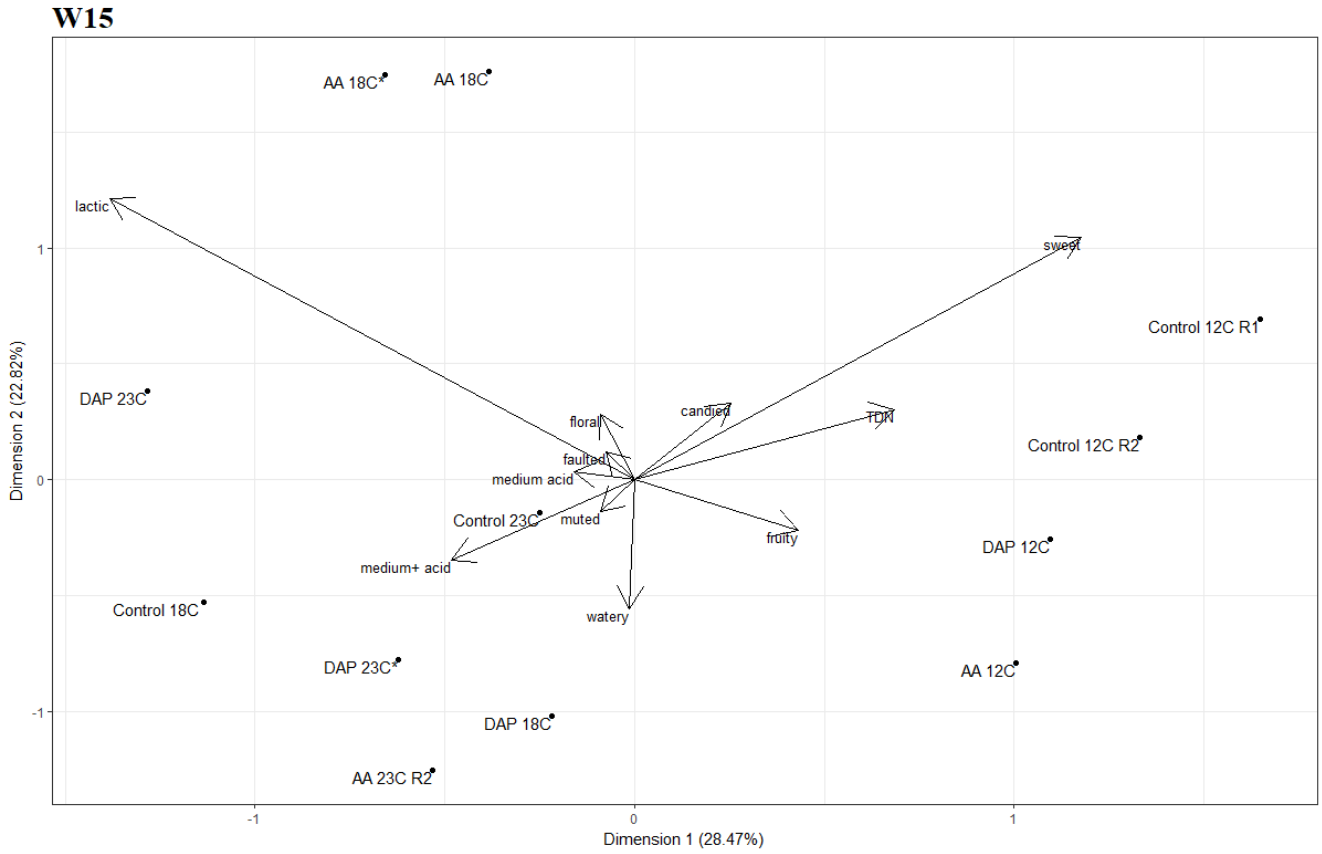


Figure 2.5. Riesling wines fermented with W15 at three different fermentation temperatures. Fermentations were received different nutrient treatments DAP to 150ppm YAN, amino acids (AA) to 150ppm YAN, or no supplementation (Control). R1 and R2 indicate different fermentation replicates.
* indicates sample replicates.

Discussion

Riesling Wine Chemistry. In this study, lower pH values were observed in wines fermented at higher temperatures (Table 2.1). Lower pH at higher temperature can be the result of increased acetic and succinic acid production, and yeast uptake of buffering potassium ions (Ough et al. 1969). Yeast uptake of potassium ions is directly proportional to increased population size and subsequent enzymatic activity (Ough, Amerine, and Sparks 1969), and there is often increased population size, particularly early in fermentation, at higher temperatures (Torija et al. 2003). The expected increase in yeast population size

at higher temperature may have resulted in an increase in potassium uptake, which would reduce the buffering capacity of the medium, accounting for the decrease in pH at higher fermentation temperature. Potassium levels were not recorded in the course of this study, however. Acetic acid production can also contribute to lower pH, but acetic acid production was lowest at 18º C (Table 2.3), and a corresponding increase in pH at 18º C was not observed (Table 2.1), suggesting that there was not a linear correlation between acetic acid production and pH. Succinic acid production from malic acid metabolism can vary according to fermentation temperature as well (Ough, Amerine, and Sparks 1969). The significant differences observed in malic acid levels (Table 2.5) may have been the result of malic acid metabolism to succinic acid in W15. This strain, which is known for higher levels of succinic acid production (Scott Labs Handbook 2016) may have produced sufficient succinic acid (not measured) to account for the observed decrease in pH and increase in TA as fermentation temperature increased.

Previous work has shown that higher YAN additions result in lower wine pH, particularly when using DAP (Torija et al. 2003, Ugliano and Herderich 2007), and in higher TA (Ough et al., 1969). Ammonium uptake results in the excretion of protons, decreasing the pH of the medium, but amino acid uptake leads to lower proton excretion (Pena, Pablo Pardo, and Ramirez 1986, Torija et al. 2003). This may account for the lower pH observed in W15 fermentations supplemented with DAP (Table 2.1), but this cannot explain the varying response to nutrient type observed in EC1118. The interactive effect of nutrient type and fermentation temperature may explain the fact that amino acid supplementation resulted in the lowest pH for EC1118.

In addition to pH and TA, nitrogen supplementation can alter acetic acid production. Nitrogen stress from either deficient or excess YAN can induce spikes in acetic acid (Vilanova et al. 2012), and excessive DAP additions can independently induce a stress-like state that causes increased acetic acid production compared to its organic nitrogen equivalent (Vilanova et al. 2007, Torrea et al. 2011). In EC1118, DAP-supplemented fermentations resulted in lower acetic acid production than the amino nitrogen treatments, and nutrient had a greater effect than fermentation temperature. The opposite is true for W15, where

fermentation temperature influenced acetic acid production more than nutrient type (Table 2.3). Acetic acid is produced by yeast to establish redox balance, and it can produce more acetic acid in response to both high temperature (Woo et al. 2014) and either high or low YAN, largely as a byproduct of glycerol formation (Vilanova et al. 2007). Both the production of glycerol (not measured) and response to temperature varied widely by strain, and these environmental stresses could explain the observed strain differences in acetic acid production.

Titratable acidity did not not always show a linear relationship between lower pH and higher TA (Table 2.2). In fact, for EC1118, TA was lower in nitrogen supplemented, higher-temperature fermentations, even though pH was also lower. In the case of W15, however, there was a more linear relationship between TA and pH, where TA was higher with higher fermentation temperature, and pH lower. The strain differences may indicate differences in metabolic activity, such as succinic acid production, which is known to vary by strain (Bell and Henschke 2005).

The observed compositional differences in wine cannot be explained by either nutrient type or fermentation temperature alone, suggesting that an interactive effect of strain, nutrient type, and fermentation temperature must be considered in order to modulate a given wine parameter during commercial winemaking.

YAN Consumption and Fermentation Kinetics. The rate of sugar consumption was higher, and time to fermentation completion shorter, than the control in fermentations supplemented to 150ppm YAN with either DAP or amino acids. Previous studies suggest that yeast have faster fermentation kinetics when supplied with amino nitrogen versus DAP, as they can better incorporate a mixture of amino acids into their required proteins for metabolic function. The supply of an array of mixed amino acids allows yeast to avoid the metabolically costly process of amino acid anabolism from a single simple nitrogen source like ammonium (Henschke and Jiranek 1993, Bell and Henschke 2005). This study, however, did not find a significant difference in fermentation kinetics in 150ppm YAN supplied from DAP versus amino acids, and the two nutrient types resulted in similar rates of sugar depletion (Figure 2.3). This may be due

to the fact that yeast uptake of nitrogen was relatively similar in this low- to moderate-YAN must, and that greater changes in nitrogen metabolism might be observed at higher YAN levels, where DAP can induce NCR and alter the pathways for amino acid uptake (Bell and Henschke 2005).

The change in kinetics as a response to fermentation temperature is also well-studied, and the observed result of slower fermentations at low temperature is likely due to the fact that lower temperatures retard both the growth rate and metabolism of yeast (Ough 1964, Fleet and Heard 1993, Karagiannis and Lanaridis 1999, Reynolds et al. 2001). The fact that fermentations at 12º C failed to reach dryness (<2g/L sugar) in all cases for W15, and for all but DAP-supplemented wines with EC1118 (Table 2.6) suggests that greater nitrogen supplementation may be required when fermenting at 12º C in order to achieve dry wine styles.

Sensory Outcomes. Based on the Napping® sensory studies, fermentations at 23º C in both strains corresponded to undesirable characteristics, including 'watery', 'reduced', and 'muted' (Figure 2.4, 2.5). This would indicate that higher fermentation temperature, though it may increase gene expression for ester production (Mouret et al. 2014), may result in fewer recognizable fruity characteristics, often ascribed to esters, in the final wine. This may be due to increased volatilization and loss from CO_2 entrainment (Mouret et al. 2014, Rollero et al. 2015). It could be compounded by the fact that increasing fermentation temperature decreases demand for unsaturated fatty acids (UFA) in yeast lipid bilayers. This decrease in demand for UFA would lead to cessation of fatty acid synthesis later in fermentation, and this would yield lower MCFA release (Waterhouse et al. 2016). Fermentations at 18º C also resulted in a perceived absence ester-based aromas in both strains, and were associated with a different set of undesirable characteristics including 'astringent', 'watery', 'oxidized', which could indicate a similar effect to that seen at 23º C. Lastly, panelists found that all of the 12º C fermentations had more fruity characteristics. While several studies have pointed to an increase in fruity ester volatiles in fermentations supplemented with amino nitrogen rather than ammonium (Hernández-Orte et al. 2005, Torrea et al. 2011), this study indicated that fermentation temperature played a greater role in the sensory outcomes of

the final wine than the nitrogen source. This result would suggest that 12º C is the more appropriate fermentation temperature for the preservation of fruity characteristics and desirable sensory outcomes in Riesling wine.

Conclusion

Nutrient type and fermentation temperature were both critical determinants for desirable outcomes in Riesling wine, and impacted final wine parameters. Wine pH, TA, residual sugar, and acetic acid were widely dependent on strain response to nutrient type and fermentation temperature, and 18º C showed the lowest acetic acid production and residual sugar of the temperatures tested, suggesting the lowest yeast stress. YAN supplementation was key to increasing fermentation kinetics, and 150ppm YAN was sufficient to avoid a stuck or sluggish fermentation, except at 12º C, which often failed to reach dryness (<2g/L RS). Nutrient supplementation type did not significantly alter fermentation kinetics, but amino acids resulted in lower residual sugar than did DAP. This would indicate that supplementation level of 150ppm YAN, which is below the current level recommended for a successful fermentation, is an adequate nutrient supply for cool-climate Riesling, when fermented at 18º C or higher fermentation temperature. Organic nutrients, like amino acids, may facilitate dryness, but did not significantly differ in fermentation kinetics or sensory outcomes. In fact, the most important determinant in sensory outcomes was low fermentation temperature, having a greater impact on sensory qualities than any supplementation type, including un-supplemented wines. While nitrogen supplementation and nutrient type has been shown to promote varying levels of important volatile compounds, this work would indicate fermentation temperature can have a greater impact on desirable sensory attributes in the final wine.

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CHAPTER 4:

EXPERIMENTAL MODIFICATIONS AND FUTURE WORK

The need for YAN supplementation is widely recognized as crucial for a healthy fermentation and the formation of volatile aroma compounds that can greatly impact wine quality. The majority of existing literature on the topic, however, focuses on warmer climate growing conditions, often with grapes and must with much higher starting YAN levels. Additionally, fermentation temperature can alter gene expression, cellular composition, and metabolism of yeast, often having an interactive effect with nutrient supplementation. This work sought to define the optimal fermentation conditions and nutritional composition for cool-climate Riesling fermentations, and added to the growing evidence that YAN requirements in cool-climate fermentations may be below currently recommended levels. Further, it provided new insight into the optimal fermentation conditions and sensory outcomes for cool-climate Riesling.

Inorganic versus Organic Nitrogen Supplementation Impacts the Chemistry and Sensory Properties of Cool-Climate Riesling.

Given the growing body of evidence that a complex mixture of nutrients is required to optimize the production of fermentation volatiles, future work should investigate the implications of adding a mixed micronutrient package in the course of nutrient optimization. Nitrogen metabolism plays a central role in the formation of higher alcohols and their corresponding acetate and ethyl esters via the Ehrlich pathway, but this study showed that nitrogen supplementation, particularly in excess of 150ppm, can have detrimental effects on wine quality. Most nitrogen is exhausted early in the fermentation in the course of biomass accumulation, and it is stored in the cell vacuole for continued metabolism and cellular maintenance. Other principle micronutrients such as biotin, thiamin, and pantothenic acid have emerged as important co-factors in yeast biomass accumulation and cellular metabolism, but relatively little evidence exists regarding the potential for further reducing or changing the need for nitrogen

supplementation in the presence of these micronutrients, which can be supplied by readily available commercial products, such as GoFerm and Fermaid A.

In an effort to improve this experimental design, one could conduct a full quantification of fermentation volatiles via gas chromatography. This would allow the study investigators to compare the impacts of nitrogen source to the formation of higher alcohols and their corresponding esters. A full list of fermentation volatiles could then be compared to the observed sensory outcomes, illuminating possible causal relationships between the formation of volatiles and the observed sensory outcomes.

Nitrogen Supplementation and Fermentation Temperature Impacts the Chemical and Sensory Outcomes of Cool-Climate Riesling. It is well known that lower fermentation temperature results in the release of mid-chained fatty acids, which can undergo acid-catalyzed esterification into ethyl esters in the presence of ethanol. Higher fermentation temperature, however, results in higher expression of the genes responsible for acetate and ethyl ester production during fermentation. Nitrogen metabolism also plays a central role in acetate and ethyl formation, so this study sought to determine the optimal fermentation conditions and nutrient source for cool-climate Riesling. Though this study revealed that lower fermentation temperature was more important than nitrogen source for perceived wine quality, this work may or may not be applicable to red wine fermentations, which are routinely much higher in order to facilitate phenolic extraction. Further work should seek to investigate the implications of fermentation temperature and nitrogen source for both phenolic extraction and perceived wine quality in red wine. Additionally, a full quantification of wine volatiles via gas chromatography could elucidate the changes in metabolism, particularly in higher alcohols and esters, which occur in different levels in response to various nitrogen sources and fermentation temperatures.